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AGRICULTURAL METEOROLOGY: SUMMER SEQUENCE OF MONTHLY MEAN TEMPERATURE AT WINNIPEG, SWIFT CURRENT, AND EDMONTON[1]

BY J. W. HOPKINS[2]

Abstract

An analysis of the monthly sequence of mean temperature during the summer period, April–September, of the years 1894–1937 has been made by expressing each annual sequence as an orthogonal polynomial function of time. Whereas the precipitation sequence, previously studied, required terms of the fourth or fifth degree for its adequate representation, the average temperature sequence at all three stations was very closely approximated by a third degree polynomial. On the average, corresponding coefficients for each station differ significantly, indicating decreasing continentality of the temperature regime with distance westward from Winnipeg. Annual variations in corresponding coefficients for the three locations are appreciably correlated, but for the most part exhibit no regular sequence in time. However, in recent years at Edmonton the mean temperature for April has tended to be lower, and that for July and August to be slightly higher, than previously. There is some suggestion of a feeble inverse correlation in the annual fluctuations of temperature and precipitation.

Introduction

In a preceding paper (3), the writer has described a statistical analysis of the monthly sequence of summer precipitation at three representative meteorological stations in the Prairie Provinces of Canada. It is now desired to present the results of a parallel analysis of monthly mean air temperature at the same locations, viz., Winnipeg, Manitoba (lat. 49° 53′ N., long. 97° 7′ W., alt. 760 ft.), Swift Current, Saskatchewan (50° 20′ N., 107° 45′ W., 2440 ft.), and Edmonton, Alberta (53° 33′ N., 113° 30′ W., 2158 ft.), and to compare certain characteristics of the temperature and precipitation sequences.

Data

As before, the primary data, namely the mean air temperatures in degrees F. recorded at each station for the months mentioned were extracted from the published observations of the Meteorological Service of Canada (1). However, whereas the analysis of precipitation sequences comprehended the period 1890–1937, temperature records were incomplete in the earlier years,

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Published as Paper No. 188 of the Associate Committee on Grain Research, and as N.R.C. No. 1016.

[2] *Statistician.*

TABLE I

SEASONAL TEMPERATURE COEFFICIENTS, WINNIPEG

Year	a'	b'	c'	d'	e'	f'
1894	57.5	93	−219	−17	15	−43
1895	56.2	51	−112	−44	0	20
1896	55.7	92	−211	17	− 3	−11
1897	57.2	141	−118	6	10	66
1898	56.0	126	−150	− 4	− 2	28
1899	55.5	121	−180	−24	6	6
1900	59.5	59	−138	−36	− 8	−54
1901	57.8	75	−164	−40	−16	64
1902	55.7	118	−169	−27	−17	81
1903	55.3	74	−173	−11	− 1	5
1904	54.3	108	−176	28	−10	2
1905	56.0	156	−126	−24	0	6
1906	58.8	121	− 98	−54	28	−36
1907	51.7	186	−223	−59	31	−37
1908	56.8	125	−140	0	14	18
1909	56.5	189	−207	19	− 5	− 7
1910	57.5	95	−174	−40	38	−44
1911	56.7	78	−169	3	− 3	−39
1912	56.3	78	−164	18	12	−18
1913	57.7	94	−121	−51	15	−75
1914	57.7	138	−193	13	9	71
1915	57.2	75	− 79	−85	−13	1
1916	56.0	144	−180	−76	6	82
1917	55.7	150	−175	−15	7	51
1918	54.8	81	−164	−94	− 2	−44
1919	59.8	95	−182	10	0	−16
1920	57.2	201	−190	26	−24	−20
1921	58.5	127	−195	17	17	−11
1922	59.7	102	−151	27	−19	− 9
1923	58.0	152	−207	57	23	15
1924	53.7	152	−154	−68	14	8
1925	58.0	112	−126	−88	0	−14
1926	56.3	92	−185	−13	−23	73
1927	56.0	132	−135	−33	15	− 3
1928	55.7	132	−184	12	−20	60
1929	56.3	126	−176	−104	4	−28
1930	58.7	124	−169	−101	11	−13
1931	58.8	143	−143	−37	19	−47
1932	58.5	125	−192	10	− 2	−52
1933	58.8	137	−200	12	2	−30
1934	55.5	83	−186	− 2	−12	32
1935	55.5	133	−195	−67	3	91
1936	58.0	174	−237	19	−11	137
1937	55.3	60	−119	5	7	19
Mean	56.8	117.5	−167.0	−20.8	2.6	6.5
Standard deviation	1.66	36.69	34.72	40.08	14.34	46.64
"t"	–	21.836**	31.905**	3.442**	1.209	0.921

** *Exceeds 1% level of significance.*

and the study of these accordingly had to be confined to the period 1894–1937. Again as before each observed six-monthly sequence was expressed by the use of Fisher and Yates' multipliers (2) as a fifth degree orthogonal polynomial function of time, the coefficients of which are listed in Tables I, II, and III.

TABLE II

SEASONAL TEMPERATURE COEFFICIENTS, SWIFT CURRENT

Year	a'	b'	c'	d'	e'	f'
1894	58.2	93	−193	−77	− 5	− 1
1895	55.5	43	−117	−97	− 1	31
1896	55.5	97	−195	−53	17	11
1897	59.0	104	−129	− 1	−23	25
1898	55.7	138	−172	−32	−12	8
1899	53.7	136	−148	−14	18	44
1900	59.0	26	−153	−29	3	−13
1901	56.3	48	−155	−87	−45	123
1902	54.7	92	−130	−28	−26	52
1903	54.2	69	−154	−46	14	−38
1904	55.0	110	−159	−15	− 1	15
1905	55.3	140	−134	−70	−10	−14
1906	58.0	98	−102	−92	16	14
1907	51.2	163	−175	−57	7	−15
1908	56.8	93	−122	−32	12	62
1909	55.0	182	−171	17	− 9	7
1910	56.3	40	−116	−50	30	8
1911	53.8	83	−152	8	6	−38
1912	55.0	52	−153	−53	3	−61
1913	57.2	81	−112	−54	14	−72
1914	57.5	103	−168	−62	14	92
1915	56.2	39	− 73	−131	−23	−37
1916	54.3	110	−131	−75	13	57
1917	56.2	131	−178	6	−16	120
1918	57.0	102	−162	−58	4	−44
1919	60.0	94	−159	−41	− 1	−19
1920	56.2	183	−193	− 7	−19	31
1921	57.0	104	−210	−26	0	−52
1922	57.5	133	−126	−12	0	−42
1923	57.0	106	−168	− 4	14	10
1924	54.5	131	−138	−34	6	22
1925	57.2	77	−136	−58	− 2	28
1926	55.7	34	−181	−81	−11	81
1927	55.0	122	−144	−58	16	−26
1928	56.5	107	−159	37	−29	83
1929	55.5	137	−192	−98	− 2	−16
1930	58.0	96	−129	−119	5	−13
1931	59.0	92	−156	−18	8	−18
1932	58.0	82	—132	−28	− 4	−20
1933	57.7	116	−190	−24	4	−30
1934	57.3	28	−173	−47	−29	77
1935	54.5	137	−168	−38	14	68
1936	59.5	119	−210	−16	−14	112
1937	59.2	101	−157	−49	5	37
Mean	56.4	99.4	−154.0	−43.2	− 0.9	14.8
Standard deviation	1.82	38.11	28.59	35.76	15.50	49.64
"t"	–	17.296**	35.726**	8.023**	0.379	1.970

** *Exceeds 1% level of significance.*

Analysis

Characteristics of Average Sequence

In contrast to the situation encountered in considering the rainfall sequence (3), the frequency distributions generated by the 44 annual values of the coefficients $a' \ldots\ldots f'$ show only two significant departures from normality,

TABLE III

SEASONAL TEMPERATURE COEFFICIENTS, EDMONTON

Year	a'	b'	c'	d'	e'	f'
1894	53.7	58	−178	−62	−16	20
1895	53.2	25	−130	−50	2	26
1896	52.7	102	−169	−13	11	19
1897	55.8	61	−110	6	−22	− 6
1898	55.5	93	−141	− 7	−17	13
1899	51.8	111	−125	1	17	11
1900	54.0	10	−114	10	− 6	2
1901	52.5	51	−147	−49	−35	55
1902	52.8	65	−110	−50	−22	62
1903	51.7	68	−157	− 7	5	−41
1904	53.7	64	−109	−41	9	17
1905	53.8	71	−104	−24	− 4	24
1906	55.8	63	− 98	−92	28	14
1907	50.2	125	−157	−15	19	− 3
1908	54.7	70	−124	−10	− 8	70
1909	52.8	147	−164	52	− 2	14
1910	53.8	47	−113	−33	11	15
1911	52.7	74	−139	9	3	− 9
1912	54.5	39	−129	− 1	− 9	−65
1913	54.3	74	− 95	−41	9	−37
1914	54.3	76	−140	−54	0	18
1915	54.5	39	− 84	−116	−28	−16
1916	52.7	76	−106	−34	4	4
1917	53.3	106	−161	41	−21	73
1918	53.3	92	−113	−53	11	−25
1919	55.2	77	−103	−53	− 1	− 7
1920	51.0	150	−198	0	− 6	72
1921	53.0	54	−147	−11	7	−13
1922	54.8	103	−128	−12	− 6	− 6
1923	54.0	94	−132	− 6	10	−12
1924	53.0	88	−144	− 2	2	68
1925	53.3	44	−146	−26	− 4	20
1926	52.3	20	−164	−80	− 2	74
1927	51.8	113	−158	−52	2	−32
1928	52.7	94	−160	54	−18	72
1929	52.5	103	−174	−12	− 4	− 6
1930	54.5	83	−135	−87	1	− 3
1931	54.2	59	−118	−46	− 4	16
1932	55.7	88	−121	− 7	−13	−23
1933	53.5	85	−147	−35	−21	−25
1934	53.0	8	−132	−42	−18	24
1935	52.3	136	−185	21	5	51
1936	54.5	93	−189	23	−21	55
1937	58.0	130	−192	−50	−16	26
Mean	53.6	77.9	−138.4	−24.0	− 3.8	13.8
Standard deviation	1.43	33.63	28.28	36.44	13.47	34.51
"t"	–	15.371**	32.460**	4.370**	1.880	2.647**

** *Exceeds 1% level of significance.*

namely negative skewness in the case of a' at Swift Current and e' at Edmonton. It may accordingly be inferred that at the former station summer seasons of moderately above and appreciably below average mean temperature have been more frequent than those moderately below or appreciably above average,

respectively. At Edmonton there has been a similar uneven occurrence, not of the mean temperature for the six months as a whole but of the element of seasonal trend corresponding to the fourth degree polynomial term ξ_4' (2). As is noted below, however, this is not a salient feature of the average season. Apart from these two instances, the annual fluctuations would seem to range themselves symmetrically about the mean in each case.

Whereas the sequence of precipitation previously examined (3) required significant polynomial terms of the fourth or fifth degree for its adequate representation, the average temperature sequence was closely approximated by a third degree function, as is illustrated in Fig. 1. At all three stations this

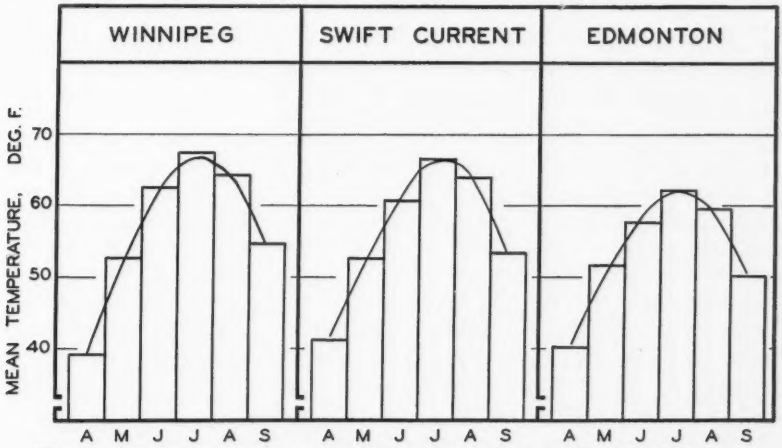

FIG. 1. *Average summer sequence of monthly mean temperature at Winnipeg, Swift Current, and Edmonton, 1894–1937 (rectangular columns), and approximation to sequence by third degree polynomial (continuous curves).*

average polynomial is characterized by a positive linear coefficient b' (arising from the fact that July, August, and September have a higher mean temperature than April, May, and June), a negative quadratic coefficient c' (monthly mean temperature at a maximum in July) and a small negative cubic coefficient d' (reflecting some asymmetry in the rate of increase and decrease in mean temperature before and after the July maximum). However, although the average seasonal trend is thus of the same general nature at each of the three locations, the analyses of variance summarized in Table IV demonstrate that the average numerical magnitude of each of the trend coefficients mentioned above (as well as that of the general mean a') differs between stations by a statistically significant amount. On the average, both b' and c' are largest at Winnipeg, smallest at Edmonton, and intermediate at Swift Current (Tables I to III). This may be associated with decreasing "continentality" of the temperature regime with distance westward from Winnipeg, the station most centrally situated with respect to the land mass.

TABLE IV

ANALYSIS OF VARIANCE OF TEMPERATURE COEFFICIENTS

Variance	Degrees of freedom	Mean square			
		a'	b'	c'	d'
Between stations	2	135.0500**	17,261.8**	9029.4**	6490.5**
Between years	43	6.1474**	3237.0**	2220.5**	3089.6**
Remainder	86	0.9743	310.1	320.7	561.2

** *Exceeds remainder, 1% level of significance.*

The average of the mean temperatures for the six months (a') at Edmonton is significantly lower than that recorded at either of the other two points.

Annual Variations

In general the temperature sequence is much more stable from year to year than the precipitation sequence. It has already been mentioned that the annual temperature coefficients are for the most part distributed at least approximately in accordance with the normal law of frequency about the mean values characteristic of each station. The standard deviation of corresponding coefficients $b' \ldots f'$, shown at the foot of Tables I, II, and III, is very similar at all locations. That of a' is greatest at Swift Current (1.82°) and least at Edmonton (1.43°), but this difference likewise is statistically insignificant. Furthermore, there is a considerable degree of association between the annual fluctuations at the three stations of each of the coefficients $a' \ldots d'$ investigated in Table IV, which accounts for from 73 (d') to 84% (b') of the total annual variance. On the other hand no significant correlation in the variation from year to year of the mean temperature for the season (a') and that of any of the sequence coefficients $b' \ldots f'$ was demonstrable at either Winnipeg, Swift Current, or Edmonton, indicating that in general the phase and amplitude of the temperature sequence are the same in seasons of below-average mean temperature as in those above-average. This is in contrast to the situation found to prevail in respect of precipitation (3).

As in the case of the rainfall coefficients previously considered, the secular trend of the temperature coefficients for each station was investigated by a further regression analysis in which a fifth degree polynomial function of time was fitted to each series of 44 $a' \ldots f'$, also by the use of Fisher and Yates' multipliers (2). With one exception the resulting regression coefficients proved to be uniformly negative, leading to the deduction of an essentially random incidence, without orderly sequence in time, of the annual variations described above. The exception mentioned is provided by the quadratic coefficient c' for Edmonton which, as illustrated in the upper part of Fig. 2, has shown a slight trend over the period of record (accounting for about 30% of the annual variance) resolvable into components tentatively ascribable to a long-term oscillation, of about 30-yr. phase, superimposed on a gradual progressive

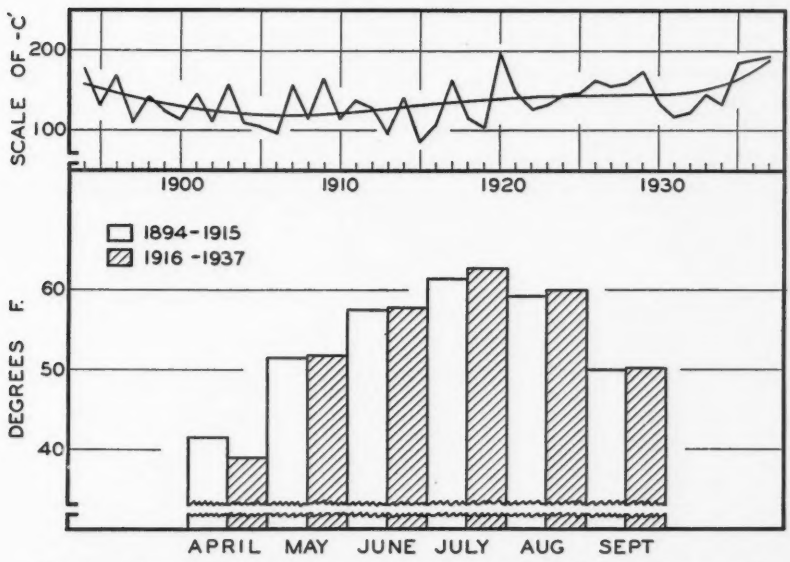

FIG. 2. *Upper portion: Annual fluctuations and secular trend of the temperature coefficient c' for Edmonton, 1894–1937. Lower portion: Average summer sequence of monthly mean temperature at Edmonton, 1894–1915 and 1916–1937.*

increase. This is reflected in the monthly mean temperatures for the periods 1894–1915 and 1916–1937, the average for April being 2.5° F. lower in the latter, whilst the averages for July and August are respectively 1.2 and 0.8° higher. In this connection, it may be remarked that Edmonton was likewise the only one of these three stations to show any progressive change in the seasonal incidence of precipitation (3).

TABLE V

COVARIANCE IN ANNUAL FLUCTUATIONS OF TEMPERATURE AND RAINFALL COEFFICIENTS

Sequence coefficient	Coefficient of correlation		
	Winnipeg	Swift Current	Edmonton
a'	+0.01	−0.33*	−0.23
b'	−0.01	−0.23	−0.08
c'	−0.05	−0.28	−0.12
d'	−0.01	−0.36*	−0.23
e'	−0.16	−0.35*	−0.32*
f'	−0.08	−0.16	+0.05

* *Exceeds 5% level of significance* ($r = \pm 0.30$).

Covariance of Temperature and Rainfall Coefficients

Covariance in the annual fluctuations of corresponding rainfall and temperature coefficients was investigated by calculation of the correlation coefficients listed in Table V. Sixteen of the 18 are negative in sign, and four may be regarded as individually statistically significant. There is thus some suggestion of an inverse relation between seasonal temperature and precipitation, at Swift Current and Edmonton at any rate, but the indications are that for the most part this is rather tenuous.

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THE ORIGIN AND HISTOLOGY OF BORDEAUX SPRAY RUSSETING ON THE APPLE[1]

By Hugh P. Bell[2]

Abstract

Apple trees of the McIntosh Red variety were sprayed at about the time of full bloom in 1939 and 1940. The origin and structure of the resultant russet tissue is described. The first apparent injury is a browning of the epidermal cells at the base of the hairs. The growth of these browned cells is inhibited and, owing to this, cracks occur as the fruit enlarges. Adjacent hypodermal and cortical tissue is exposed and killed. Cork cambiums and cork are formed in the cortex. This cork is different in origin from normal russet cork, which originates in the epidermis. The further enlargement of the fruit causes the cracks to multiply, extend tangentially, and deepen. All tissues external to the innermost point of fissure penetration become killed. The final scurf-like patches of scar tissue are a mixture of dead epidermis, hypodermis, cortex, cork, and cork cambiums. This scar tissue is not true cork.

Introduction

As there is an appreciable annual loss of apples from russeting induced by spray materials, it was considered advisable to obtain information regarding the origin and histology of the russet tissue appearing on fruit sprayed with Bordeaux mixture under field conditions. The investigation reported below was undertaken to provide this information.

Preliminary Studies

The morphology of abnormal tissue can be studied most effectively by comparing it with normal tissue. With this in mind, the development of the protective layers of both the McIntosh Red (2) and the Golden Russet (3) were worked out in detail. These normal forms of development were used as a basis for comparison in this study of the abnormal tissue of induced russeting.

Material and Technique

The material for the study was obtained from trees of the McIntosh Red variety, at the Dominion Experimental Station, Kentville, N.S. The trees were sprayed with Bordeaux mixture as follows: in 1939, on May 31 and again on June 7; in 1940, on May 22 and again on June 11. The date of full bloom for the McIntosh Red at Kentville in 1939 was June 8 and in 1940, June 4. In both years this treatment provided an ample supply of russeted fruit.

From the day the spray was applied, the material was collected twice each week in the early part of the season and once a week during the latter part of the season. This material was treated in two ways. Part was killed in

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Contribution from the Department of Biology, Dalhousie University, Halifax, N. S., with financial assistance from the National Research Council of Canada.

[2] *Professor of Botany.*

chromacetic, imbedded in paraffin, and examined in the form of serial sections that had been stained with safranin and fast green. The other part of the material was cut while still fresh and the free-hand sections were mounted in glycerine jelly. For the identification of the injured tissues, especially the early stages, the free-hand sections mounted in glycerine jelly were the more valuable for in these the original brown discoloration could be seen in sharp contrast to the green or colourless normal cells. To detect the very early stages, tangential sections are the best. The most satisfactory way to obtain these is to use a sharp safety razor blade and do the actual cutting under the lower power lenses of a binocular dissecting microscope. In this way a section can be obtained that contains little more than the epidermal layer.

Development and Histology

During the period immediately following the application of the spray, a large amount of material was examined very carefully, but injury to the tissues was not observed until from 10 to 15 days after the first application. This agrees with the findings of Young and Walton (9, p. 412) who state "a long period may elapse after the application of a copper spray before the injury appears". When the first signs do appear they develop almost simultaneously on a large percentage of the young fruit. This first indication of an injury is a conspicuous browning of the epidermal cells and is quite easily identified by means of the tangential sections of fresh material. At first this browning occurs only in the epidermal cells immediately surrounding the hair base (Figs. 1 and 3), but very shortly it spreads to the adjacent epidermal cells (Fig. 4) and to the hypodermal layer immediately below the epidermis. Almost immediately a series of tangential divisions occur in the hypodermal cells beneath the hair. This elevates the injured tissue slightly above the remainder of the epidermis (Fig. 2). Except for the fact that these divisions occur in the hypodermis and not in the epidermis, they are similar to those that normally precede the formation of a cork cambium in the Golden Russet apple (3, p. 564, Figs. 10, 11), but these cambium initials below the injured tissue form merely patches of cork and these are seldom more than three or four cells in thickness, for, as will be described later, the cambium cells are almost immediately inactivated by the further progress of the injury.

Occasionally a similar browning may be observed in the cells surrounding a stoma (Fig. 5), but in most cases the guard cells and the adjacent epidermal cells are unaffected. When they are browned, the progress of the injury is similar to that which has been and will be described for the injury originating at the hair base.

At the time browning first appears, there is some evidence that the application of the spray may stimulate the activities of some epidermal cells and more definite evidence that it inhibits the activities of others. The apparent *stimulated activity* occurs in some of the epidermal cells adjacent to, but not in, the injured area and consists of a single tangential division (Fig. 9). This is exactly similar to the first division in the formation of a normal periderm in

the Golden Russet apple (3, pp. 561 and 562, Figs. 1 to 6). In the McIntosh Red fruit injured by Bordeaux spray, this activity does not proceed beyond the formation of one tangential wall. Such tangential division of the epidermal cells has not been observed by the author in the normal development of the McIntosh Red. This of course is only negative evidence and the number of observations that can be made by any one investigator is limited; also no controlled experimental work regarding the appearance of the wall was attempted, so there is no proof that stimulation occurred; but, in the material examined from both normal and sprayed fruit, the evidence as outlined above appeared to suggest some association between the application of the spray and the formation of this single tangential wall. The *inhibition of normal activity* occurs in the epidermal cells that are browned. To see this, an injury at the stage illustrated in Fig. 2 must be examined in a stained microtome section. In such a mount the cell contents are not masked by browning as they are in the hand sections mounted in glycerine jelly, consequently it can be seen that the affected epidermal cells have normal nuclei, unplasmolysed cytoplasm, and a cuticle that has thickened uniformly over the whole surface. These cells are usually conspicuous, owing to their raised position, but whether they are or are not raised, they may be identified by their shape, for in their development they have not kept pace with the unaffected epidermal cells. These go through a regular sequence of rapid changes in shape and size as the fruit starts to enlarge (2, p. 396), but during the two weeks subsequent to the application of the spray the affected or browned cells remain unchanged so far as shape and size are concerned. This suggests that the normal growth of the affected cells was inhibited about the time that the spray was first applied. Thus the application of Bordeaux spray about the time of full bloom may have some relation to the appearance of a tangential wall in certain epidermal cells and it apparently inhibits the normal development of other epidermal cells, namely, those that are turned brown.

A few days after the first appearance of the injury, cracks develop on the surface of the young fruit. These usually appear at about the centre of the browned areas and after extending inward through the epidermis and dividing hypodermal cells, they expose what had, up till then, been unaffected cortical cells (Figs. 6, 7). These cortical cells immediately turn brown and at the same time the browning effect spreads to all the cells in the immediate vicinity. The whole lesion in cross section is now a lens-shaped mass of browned tissue with the centre of its inner convex surface at the point to which the fissure penetrates most deeply (Fig. 8). During the inward progress of the browning, the cork cambium initials, which had been formed beneath the hair base, collapse and become indistinguishable. The injured epidermal, hypodermal, and cortical cells turn a very dark brown and collapse. The disintegrated cell contents are deposited on the cell walls, giving to these walls the appearance of being very thick. Again, and in limited patches just inside the lesion, the initial stages of a secondary cambium frequently develop (Fig. 9), but sometimes there is no evidence of such cambium formation

(Fig. 8). In the latter case many of the cortical cells immediately inside the lesion may elongate radially or some of the large cortical cells may divide by a tangential wall (Fig. 7), but these tangentially divided cortical cells are not necessarily arranged in any definite layer. They may be scattered at random among the cells just inside the injury. These secondary activities of both cambium formation and tissue proliferation are early inhibited for the cells involved are very soon both exposed and isolated as a result of the still further inward penetration of the fissure.

The subsequent development of the injury is probably brought about by the enlargement of the fruit and there is a continuous repetition of the process described above. That is, the fissures deepen, the browning effect extends to all adjacent cells, and cork cambium formation and other induced activities are initiated, but almost immediately inhibited by the continued deepening of the fissures. While the injury is penetrating into the cortex, the cracks lengthen rapidly in a tangential direction on the surface. Also new cracks develop in the browned tissue on each side of the original ruptures. These various splits criss-cross like a fine network over the surface of the apple. The result is that, in the mature fruit, the injury consists of many small, medium, and large cracks, which are separated by browned, dried masses of epidermal, hypodermal, and cortical tissues mixed with scattered patches of cork (Fig. 10). It is these brown scurf-like masses of dead cells that give to the injury its russeted appearance. Russet tissue of this type may be found during late June or early July and except for becoming thicker and more extensive, it does not change during the subsequent development of the fruit.

The mixed composition of this tissue is brought out clearly by microchemical tests. The patches of cork give the reactions typical for suberin. Enclosed between these cork layers, there are groups and layers of dead parenchyma cells, the walls of which give the typical cellulose test. There are also many cells that are parenchyma-like in shape, but with walls that react for neither suberin nor cellulose. The exact chemical composition of the walls of these cells was not determined. A cuticle, associated with collapsed epidermal cells, still adheres in places. When present it is easily differentiated by the usual tests. Thus, by this means, four or five different types of tissue may be identified in the lesion.

The method by which the spray penetrates the cell was not determined, but observations were made that furnish negative information. Attention was directed to this problem by Horsfall and Harrison (5, p. 441) in their paper dealing with Bordeaux injury to the tomato. They put forward the theory that the spray "saponifies the cuticle". Having this statement in mind all material was examined very carefully to see if any evidence could be found to suggest that this theory was applicable to Bordeaux injury on the apple. It is not difficult to make observations on this point for, at the time the apple is in full bloom, the cuticle over the cells immediately surrounding the hair base is slightly thicker than that over the average epidermal

cell (2, p. 394, Fig. 4), thus it is quite easily seen in hand sections of fresh material. All the sections studied indicate that at the time the injury first appears the cuticle is still intact, it is of normal thickness, and it does not show any sign of having been corroded in any way. That is, there is no morphological evidence suggesting saponification of the apple cuticle by Bordeaux spray.

Discussion

This russeting induced by Bordeaux spray is different in both origin and structure from that developed as a normal healthy growth on such varieties as the Golden Russet. The origin of the secondary cambium of normal russeting is in the epidermis (3), but in induced russeting, the secondary cambium originates in the hypodermis or cortex. In structure, normal russeting is a homogeneous cork tissue, but induced russeting is a mixture of dead epidermal, hypodermal, and cortical cells plus patches of cork.

From a histological standpoint it is not correct to use the word "cork" to designate this scar tissue of induced russeting. It is more like *rhytidome*, but it has been pointed out that one could not use this term instead of cork since rhytidome, as defined by Eames and MacDaniels (4, p. 212), connotes alternate layering of cork and dead cortical or phloem tissue, whereas the scar tissue of the sprayed McIntosh Red apples is a *mixture* wherein there may not be any cork formation. In an exact botanical description it would be best to call it merely "scar tissue". Of course in popular phraseology the term "cork" is so well established as descriptive of this tissue that it will probably always be used in papers that are not purely scientific and histological.

In the literature there are a number of descriptions of induced russet tissue on apples and in these descriptions most of the investigators refer to this russet tissue as "cork". For instance, Baker (1, p. 78) in his study of this injury states:— "This russeting consists of a corky covering to take the place of the normal cuticle and epidermis". Also Verner (8, p. 817), in discussing what is apparently a similar lesion, describes it as follows:— "A layer of cork cambium assumes the position normally occupied by the epidermis, cutting off to the outside several, or many, tangential layers of cork cells, which constitute the scurflike russet". In a paper by MacDaniels and Heinicke (6) there is a record of russet tissue that is altogether cork. They consider that frost was the cause of the injury and they both describe and figure the lesion as a typical periderm. In a case of russeting observed by Tetley (7, p. 165), she describes the tissue as "several layers of dead cells" and goes on to state that these are "above a continuous layer of cork and the meristem layer from which it is derived". The first part of her description (but not the latter part regarding a continuous cork and meristem layer) would be applicable to the Bordeaux injury under investigation. According to the statements of these and other investigators, induced russet tissue on apples may at times be only true cork, and hence quite different from that produced by Bordeaux spray on the McIntosh Red.

Why do the initial breaks in the surface occur wherever the cells have been browned by Bordeaux? The answer to this question is of importance for, after the original fissures are formed, the later stages of the injury follow automatically with the enlargement of the fruit. It cannot be said that these discoloured cells are immediately killed, for as stated above they still have a normal nucleus, an unplasmolysed cytoplasm, and a cuticle that continues to thicken; according to Verner (8, p. 820), however, the death of the epidermal cells would not be a necessary prelude to the formation of a crack. His statement on the subject is as follows:— "the problem of cracking in the apple involves inability of the peripheral region to stretch or grow as rapidly as it should when the fleshy portion of the fruit is enlarging at an abnormal rate". That is, the inhibition of tangential growth at the surface would be all that is necessary to cause fissures to form through the surface layers. From the evidence submitted above, it is apparent that such an inhibition does occur in the browned epidermal cells. This being the case, the epidermis cracks because the Bordeaux spray has inhibited its normal growth.

Acknowledgments

The author wishes to express his indebtedness to the Pathologist-in-Charge, Laboratory of Plant Pathology, Kentville, N.S., for laboratory space during two summers. The figures were drawn by Miss Elizabeth E. Bligh of Kentville.

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EXPLANATION OF FIGURES

Magnification: Figs. 1 to 9, 240×; Fig. 10, 130×.

FIG. 1. *June 12. Section through a hair base with the browned epidermal cells at the base of the hair.* FIG. 2. *June 12. Section of browned area before the epidermis has ruptured. A secondary cambium is formed.* FIG. 3. *June 17. Surface view of the stage illustrated in Fig. 1.* FIG. 4. *June 27. Surface view of the stage illustrated in Fig. 2.* FIG. 5. *July 2. Surface view of guard cells and adjacent epidermal cells turned brown by Bordeaux spray.* FIG. 6. *June 27. Section of browned cells just after the surface has ruptured.* FIG. 7. *June 24. Section of a stage slightly more advanced than that illustrated in Fig. 6. The fissure has broadened and the first formed cork cambium cells have died and collapsed. The radial arrangement of the cortical cells suggests tangential division in that tissue.* FIG. 8. *July 2. Section of a fairly advanced stage in which no secondary cambium is evident. Some of the cortical cells are elongated and radially arranged.* FIG. 9. *June 26. Section of a fairly advanced stage in which a definite secondary cambium has been formed. Two epidermal cells on the left of the section have divided by a tangential wall.* FIG. 10. *July 6. Section of a mature lesion. The fissure on the left is penetrating into the cortex in advance of cambium formation. The fissure on the right is just penetrating through a cork cambium. The outer tissue of the lesion is so collapsed and crushed that its cell structure is indistinguishable.*

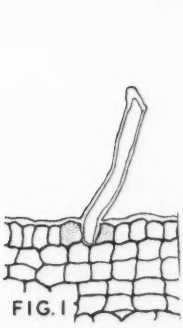


FIG. 1

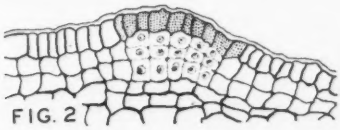


FIG. 2

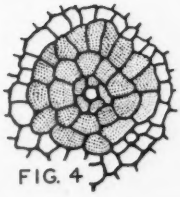


FIG. 4

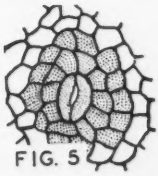


FIG. 5

FIG. 3

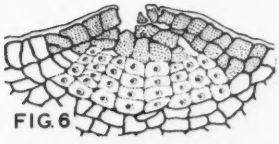


FIG. 6

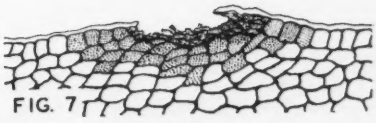


FIG. 7

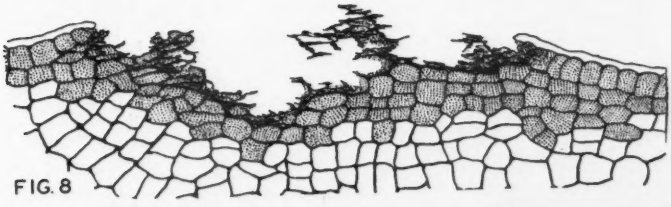


FIG. 8

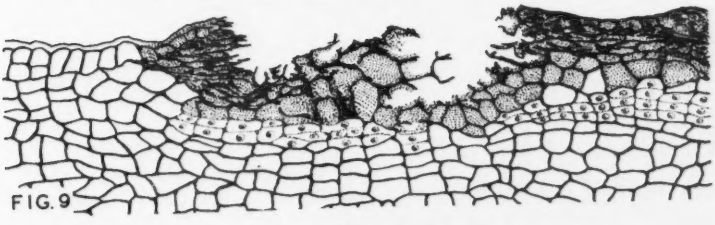


FIG. 9

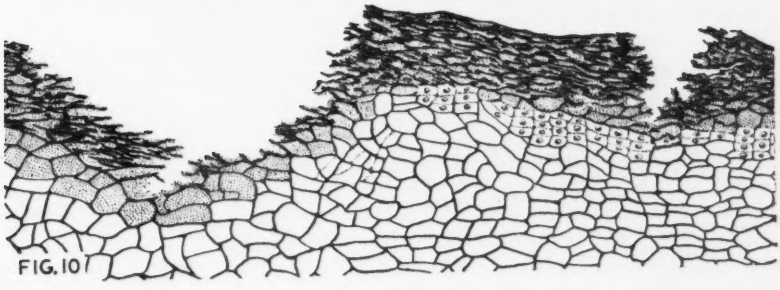


FIG. 10

STUDIES OF THE TOMATO IN RELATION TO ITS STORAGE

II. THE EFFECTS OF ALTERED INTERNAL ATMOSPHERE UPON THE RESPIRATORY AND RIPENING BEHAVIOUR OF TOMATO FRUITS STORED AT 12.5° C.[1]

By K. A. Clendenning[2]

Abstract

When a mature green tomato fruit is stored at 12.5° C. either with or without its stem, the expected respiratory climacteric accompanies the colour change associated with ripening. When the stem is removed and the stem scar area is covered carefully with hot paraffin wax, the fruit thereafter ripens slowly with a low, relatively constant rate of carbon dioxide output. These characteristics are ascribed to "auto-gas" storage resulting from restricted diffusion at the stem scar. The effect of waxing is reversible within limits since removal of the artificial seal after one month has resulted in a return to normal ripening and respiratory behaviour.

When yellowing, yellow orange, and full red fruits are stored either with or without their stems, they complete in storage those phases of the respiratory climacteric that had not been completed before detachment from the plant. The careful waxing of fruits picked at these stages of maturity inhibits further coloration and reduces the rate of carbon dioxide output to the same extent as in fruits waxed at the mature green stage. The respiratory drift of fruits picked and stored unwaxed at the early "growing green" stage is characterized by two distinct peaks. Such fruits eventually ripen and the second peak is associated with the colour change that accompanies ripening. Similar fruits stored with stem scars waxed fail to ripen before their pathological "death" and their respiration rate is reduced by the waxing treatment.

When yellowing and yellow-orange fruits are waxed, they become soft and highly susceptible to fungal attack before their ripening coloration has been completed. To inhibit the softening process in stored tomatoes, it thus appears to be necessary to apply wax before ripening has commenced. Unwaxed fruits become highly susceptible to fungal wastage only after attaining full ripeness. Waxed fruits on the other hand are subject to fungal wastage when green or partially coloured as well as when fully ripened. This is attributed to the progress of softening in the absence of the usual colour change associated with ripening. Waxing of the stem scars does not act as a deterrent to storage moulds at the waxed area. The waxed tomato has been found to be subject to several physiological disorders the symptoms of which are described.

Walford (16) observed two physiological types of tomatoes when fruits, picked at the "mature green" stage, were stored at 12.5° C. The fruits of late spring and summer showed a distinct rise in respiration rate as they ripened in storage while the late autumn and winter fruits exhibited a marked durability, colouring slowly and unevenly without an attendant rise in carbon dioxide output. He designated these contrasting types as "conventional" and "anomalous", respectively, and provisionally concluded that their distribution was related to seasonal factors.

[1] *Manuscript received July 18, 1941.*

Contribution from the Department of Horticulture, Ontario Agricultural College, Guelph, Ont., with the co-operation of the Department of Botany, University of Toronto, Toronto, Ont. This paper constitutes part of a thesis submitted to the Graduate School of the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as Paper No. 72 of the Canadian Committee on Storage and Transport of Food.

[2] *Formerly with the Department of Horticulture, Ontario Agricultural College, Guelph, Ont. Now Biochemist, Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada.*

At this laboratory, information has been accumulated on the distribution of these divergent types in relation to seasonal and experimental light conditions. Both types have been found at all seasons of the year as well as under different light intensities in the same season. Also, intermediate types appeared sporadically and they, too, had no apparent association with any special growing conditions. The preponderance of anomalous types during the winter season or under simulated winter light conditions and of conventional types during the summer in certain phases of this work suggested that, as Walford supposed, the seasonal light factor might influence the distribution of types actually present at all seasons. The complete absence of confirmatory evidence in bulk storage trials, however, demonstrated that the seasonal influence, if operating at all, was overshadowed by other determinants.

Singh and Mathur (15) reporting on the tomato fruit in relation to storage temperature stated that some of their fruits, when picked at the mature green stage passed through the ripening colour change in storage without the usual rise in respiration rate. The record submitted as typical of these was obtained at 5.7° C.; it exhibited the low, steady respiration rate and slow colour changes that characterized Walford's anomalous type.

The anomalous type is not limited to preclimacteric picks since fruits detached from the vine at the yellow and yellow orange stage frequently have fallen into this category. And further, Walford submitted records of "early growing green" fruits that showed a distinct climacteric when the colour changes occurred. The conventional type therefore was by no means restricted to fruits picked at late, and the anomalous type to fruits picked at early stages of maturity.

Examination of unpublished data available at this laboratory showed that the solution of the problem did not lie in the field of genetics since conventional and anomalous types had been obtained from the same plant. Similarly, the presence of both anomalous and conventional types within visibly uniform populations of plants or in the product of a single plant would indicate that nutrition is not the determinant of physiological type.

Nevertheless, the possibility of a relation to certain aspects of mineral nutrition has been explored incidentally by co-workers in this laboratory. The author is indebted to them for permission to refer to their results. Populations of plants were grown in parallel series receiving different amounts of nitrogen, phosphorus, and potassium, and supplied with various kinds and amounts of organic matter. Concurrent soil tests and the appearance of the plants showed that the populations were actually receiving different amounts of the nutrients named. Under all treatments the anomalous type tended to predominate with conventional types appearing only occasionally.

Phillips (13) has drawn attention to certain differences in the respiration records of fruits grown under three boron treatments. Under Walford's classification his "low boron" fruit would be designated conventional. The

"excess boron" fruit evidently was picked after the senescent rise in respiration but the pitch of its respiratory activity and its early breakdown would place it in the same category. Assuming that his "medium boron" fruit ripened in the recorded storage period it would be classed as intermediate since its respiration rate is higher than our arbitrary upper limit for anomalous records. On the basis of these observations Phillips concluded that medium boron treatment had a steadying effect on carbon dioxide output and that appropriate boron administration would lead to a low respiration rate conducive to better keeping properties. However, the diversity of types recorded by him in relation to boron nutrition is not as great as the range found in more extended investigations employing populations receiving a constant fertilizer treatment (15, 16). From this it must again be concluded that mineral nutrition is not of primary importance in the production of the physiological states of the tomato fruit with which this paper deals.

The present paper includes a survey of the relation of physiological types to season and to the internal atmosphere of the fruit as altered by waxing the stem scar. Finally, undesirable accompaniments of waxing are discussed with relation to the possible commercial application of this technique.

Materials and Methods

The populations of *Lycopersicon esculentum* Mill. var. Grand Rapids employed in this work were grown in summer outdoor plots, both shaded and unshaded, and, by accepted commercial methods (16), in greenhouse benches at all seasons of the year.

The fruits were detached from the vine at the stages of maturity indicated. After removal of the stems, the fruits were weighed individually and stored at 12.5° C. within two hours of harvesting. Fruits stored in trays were well ventilated and the relative humidity of the storage atmosphere was held between 80 and 85%. Fruits stored in respiration chambers were subjected to a steady stream of carbon dioxide free air whose relative humidity was maintained at approximately 85% by bubbling through 18% potassium hydroxide. Measurements of carbon dioxide output were made by the usual Pettenkofer method employing absorption periods of 24 hr.

Walford found that moulds developed quickly on unprotected stem scars under conditions of high relative humidity. To overcome this, he applied wax to the stem scars as this seemed to offer some protection. This practice had not been followed in earlier investigations (1, 7) in which only conventional respiration types were observed. In subsequent work (13, 15, 16) in which the stem scars were covered with wax, anomalous respiration types occasionally appeared. In the earlier part of the present investigation the stem scars were covered with wax. Subsequently the waxing was deliberately varied to extremes as noted in the text.

Results

The Distribution of Physiological Types in Relation to Season

During the year 1938 a survey of the effect of season on the distribution of physiological types was carried out by studying the respiration of individual fruits picked at the mature green stage in January and June. It was expected from Walford's work that the January fruits would be all anomalous and the June fruits all conventional. Actually the January fruits were all conventional and the June fruits gave a mixture of anomalous, conventional, and intermediate types.

The Distribution of Physiological Types in Relation to Light Treatment

In 1939 a comparison was made of the types of respiration exhibited by fruits picked at the mature green stage from summer populations grown simultaneously under three light treatments using *(a)* greenhouse benches under ordinary window glass, *(b)* a bench out-of-doors unshaded, *(c)* a similar outdoor bench shaded with cloth to 50% light intensity.

As is shown in Table I, the fruits of these three plots were a mixture of physiological types—anomalous, conventional, and intermediate records having been obtained from each population. Further, the anomalous type predominated in each plot, the conventional and intermediate types appearing sporadically.

TABLE I

THE DISTRIBUTION OF PHYSIOLOGICAL TYPES IN RELATION TO LIGHT TREATMENT

Plot	Physiological type		
	Anomalous	Conventional	Intermediate
Summer greenhouse, 1939	10	3	3
Outdoor unshaded, 1939	9	2	2
Outdoor shaded to 50% light intensity, 1939	12	1	1

Physiological Types in Relation to the Artificial Stem Scar "Seal"

Brooks (3) maintains that the gaseous exchange of the tomato fruit is principally through its stem scar; he attributes this to the relatively impervious nature of the fruit at other parts of its surface. He finds that restriction of gaseous interchange at the stem scar has an unmistakable effect on the time required for ripening. Waxing the stem scar in itself doubles the time that mature green fruits can be held at 70° C. before ripening occurs. On the other hand, application of wax to the remaining surface of the fruit with the stem scar left unsealed has little effect on the rate of coloration. He supplements these interesting observations with measurements of the carbon dioxide content of the internal atmosphere. Fruits stored with stems attached have a measurably higher carbon dioxide content than fruits with the stems removed

and they have a slower ripening rate. He also shows that a marked alteration of the internal atmosphere of the fruit results from waxing the stem scar, the concentration of carbon dioxide within the fruits so treated being twice that of the checks, over a wide range of temperatures.

Retardation of the ripening process by restriction of gaseous diffusion at the stem scar has been observed at other laboratories. Wardlaw and McGuire (17) have noted that fruits stored with stems attached ripen more slowly than similar fruits with stems removed. At this laboratory it has been found that waxing the stem scar is much more efficient in retarding the ripening process than is the practice of merely allowing the stems to remain.

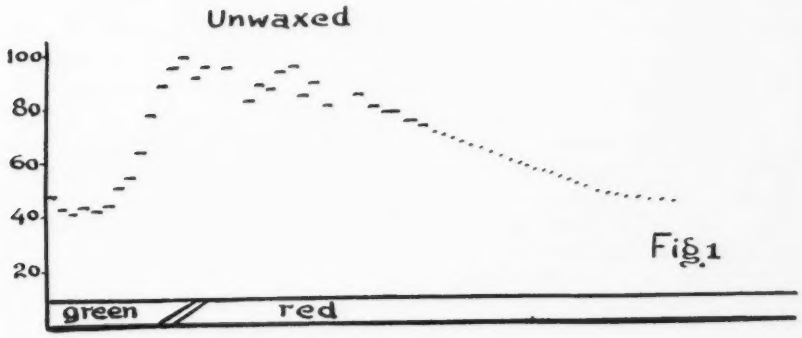
If these alterations of rate of ripening are to be attributed to altered internal carbon dioxide and oxygen concentration, the production of similar internal atmospheres by other means should have a parallel effect. Kidd and West (10) have stored tomatoes in artificial gas mixtures and they report that the colour change associated with ripening is retarded by both a decrease in oxygen and an increase in carbon dioxide of the storage atmosphere. Gustafson (8) offers further evidence on this point in finding that tomatoes stored in air ripen more rapidly than similar fruits kept in sealed containers.

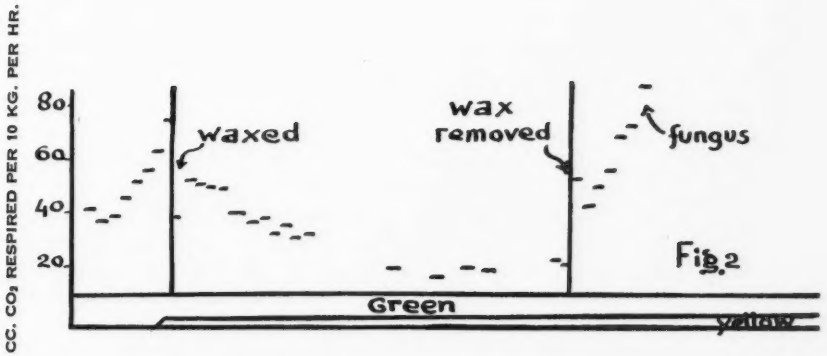
From this it would appear that any storage practice causing a marked increase in the carbon dioxide or decrease in the oxygen content of the internal atmosphere of the tomato fruit will lead also to the slow ripening rate that so constantly accompanies the anomalous type of respiration. In the work reported here, the respiration of mature green fruits has been studied in the waxed and unwaxed condition; 18 fruits were stored in individual respiration chambers with stem scars sealed carefully by a copious application of hot paraffin wax and 24 fruits were stored with stem scars untreated. *In every instance the carefully waxed fruits were of the anomalous type whereas the unwaxed fruits were always conventional in their ripening and respiratory behaviour* (Figs. 1, 5).

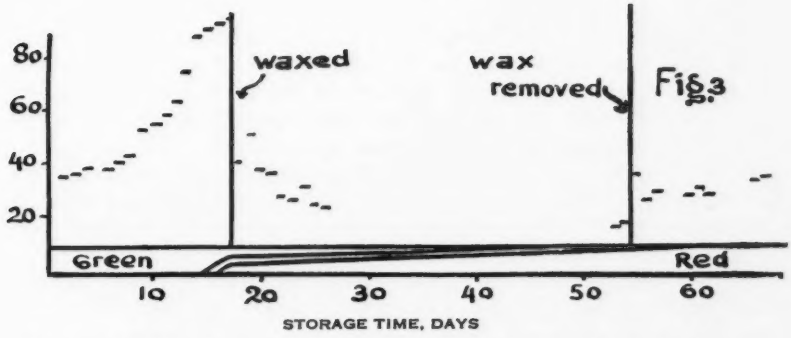
In earlier respiration studies in which the anomalous type has appeared (13, 15, 16), waxing the stem scar was practised as a matter of routine. Evidently no particular care was taken to control rigorously the effectiveness of the waxing. As a result a mixture of physiological types appeared among the waxed fruits.

The cases of greatest interest were those in which a serious restriction of gaseous interchange at the stem scar resulted from the waxing treatment. Undesirable accompaniments were usual and these are discussed in detail below. However, in some cases the sealing of the stem scar with wax has occasioned a highly desirable "auto-gas storage" of the fruit, the fruit itself inducing by its own respiration those changes in internal carbon dioxide and oxygen tension that lead to a greatly enhanced storage life.

In some instances the wax cover must have resulted in a partial seal at the stem scar so that the respiration and ripening rate was inhibited to some extent. The occurrence of the intermediate type is attributed to such a partial success in sealing.







FIGS. 1 TO 3. *Effect of waxing on respiration rate of mature green tomato fruits in storage.*

Occasionally, placing a drop of wax on the stem scar had little effect on gaseous exchange at this area, doubtless because a "seal" was not effected. This could be expected fairly consistently where, for instance, the wax was at a relatively low temperature at the time of application. As a result the "conventional" characteristics would be observed.

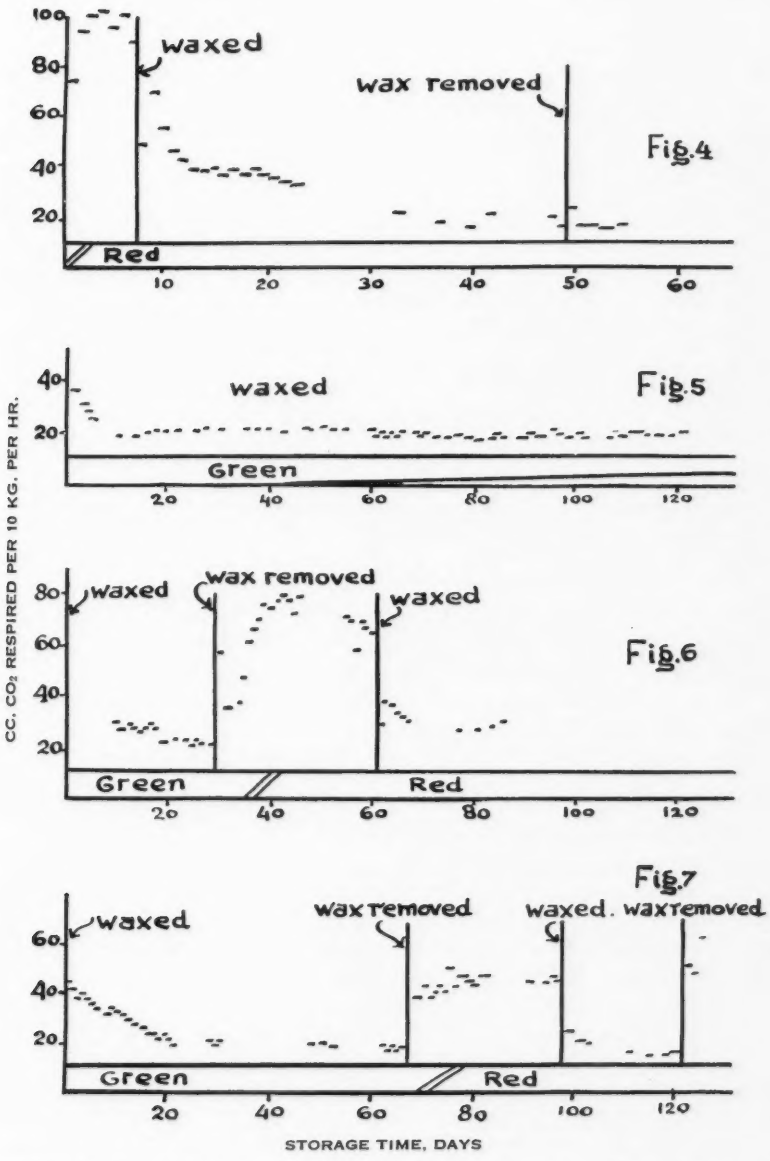
Ascribing the anomalous or auto-gas stored type to altered internal atmosphere, the question next arises as to the feasibility of changing the anomalous to the conventional type simply by removing the wax from the stem scar area. Accordingly fruits were picked at the mature green stage, the pedicels were removed, and the fresh weight of the fruit recorded. The stem scar areas then were filled with hot paraffin wax. The fruits were stored immediately in respiration chambers at 12.5° C. and their respiratory rate followed (Figs. 6, 7, 8). The respiration rate fell to low steady values because of the restriction of gaseous traffic at the stem scar area. After different periods in the "sealed" state the wax was removed and the respiration measurements continued. The fruits from which the wax was removed after about one month's storage then showed the ripening and respiratory behaviour that characterizes the conventional type. When the respiration in this "unsealed" state was declining slowly but steadily from the peak observed in the midst of the colour changes, the stem scar area was once again waxed copiously with paraffin. Thereupon the respiration rate decreased to the same level as was observed before the wax was removed.

In one instance (Fig. 6) the rate of carbon dioxide output of a carefully sealed fruit was followed for one month. Then, before there was any evidence of external ripening coloration, the wax was removed. The high value observed immediately after wax removal is attributed to the liberation of accumulated carbon dioxide. Thereafter the carbon dioxide output is taken to be truly indicative of the respiratory activity of the fruit. A typical senescent rise in respiration was observed as the fruit ripened. Thirty-one days after wax removal, wax was once again applied carefully to the stem scar and thereupon the respiration of the fruit declined to the level observed before the wax was first removed.

Fig. 7 is typical of the records obtained when the wax was allowed to remain for two months or more during which time the fruits ripened partially. Wax removal in these cases resulted in an increased rate of carbon dioxide production but the rate after wax removal was lower than in fruits from which the wax was removed after relatively short storage periods.

In order to establish the effects of waxing the stem scars of fruits picked at the mature green stage and stored for some time before wax application, the following experiment was conducted. The rate of respiration was followed in a series of such fruits stored in the unwaxed condition. Then, when various stages of ripeness had been attained, wax was applied carefully to the stem scars; Figs. 2, 3, and 4 are typical of the records obtained.

Fig. 2 shows the typical respiratory changes observed when wax was applied just as the coloration associated with ripening was becoming externally visible.



FIGS. 4 TO 7. *Effect of waxing on respiration rate of mature green tomato fruits in storage.*

The results of wax application at the peak of the respiratory climacteric is shown in Fig. 3. Wax also was applied to fruits that had ripened completely in storage; the accompanying respiratory changes are shown in Fig. 4.

These figures show that fruits of the conventional type can be made to exhibit all the respiratory characteristics of Walford's anomalous type simply by sealing the fruit at its stem scar after any stage of ripeness has been attained in storage.

After periods of one month or more in the "sealed" condition, the wax was removed from the stem scars of these fruits. Usually wax removal resulted in a reversion to respiration rates of the order found in unwaxed fruits stored for a similar length of time. However, divergences from this did occur and Figs. 2 and 4 represent the extremes observed.

The transitional changes in carbon dioxide output, shown immediately after wax application and again on its removal, have been consistent features of these waxing experiments in all but a few instances. For example, in Fig. 6, immediately after wax removal the initial reading is high because of the liberation of accumulated carbon dioxide but this complication is evidently absent after the first day. Thereafter the carbon dioxide output is truly indicative of the respiratory activity of the fruit.

On applying wax to the stem scar a month later, the first respiration reading was low because of the resulting restriction of carbon dioxide emission at the stem scar area. The succeeding values were higher because of the mounting internal carbon dioxide concentration and hence increasing diffusion through the skin. The rate of emission then tended to fall, through the depressing effect of accumulated carbon dioxide on the respiratory mechanism.

Thus on waxing the stem scar of the tomato fruit there is first, a period of rapid carbon dioxide accumulation within the fruit and a low rate of emission; secondly, a period in which internal carbon dioxide content is high and rate of both carbon dioxide production and emission falls off; finally, there is an equilibrated phase in which the rate of carbon dioxide emission is almost if not completely constant.

The final stage must be associated with a lower internal carbon dioxide concentration than exists in the second phase if the rate of gas emission is a function of its internal tension. In this connection Claypool (6) found the carbon dioxide content of completely waxed fruits to be high initially and to decrease during prolonged storage.

These changes in ripening and respiratory behaviour, as induced by wax application and wax removal, are attributed to the accompanying changes in the internal atmosphere of the fruit. This is supported by analyses of extracted gas samples from fruits stored for short periods with and without wax on the stem scars (Table II).

In making these determinations the extraction method of Brooks (2) was followed. Carbon dioxide and oxygen estimations were made on the standard Haldane gas analysis apparatus after suitable dilution with nitrogen.

TABLE II

THE EFFECT OF WAXING ON THE COMPOSITION OF THE INTERNAL ATMOSPHERE OF THE TOMATO FRUIT

Treatment	Storage time, days	Maturity of fruit	Air extracted in vacuum, 3 min.	
			CO_2, %	O_2, %
Unwaxed throughout	1	Mature green	7.0	19.3
Unwaxed throughout	8	Mature green	7.45	23.6
Unwaxed throughout	9	Colouring	12.35	19.2
Waxed initially	8	Mature green	26.6	12.8
Wax removed on 9th day, analysed 1 day later	10	Mature green	8.9	22.1

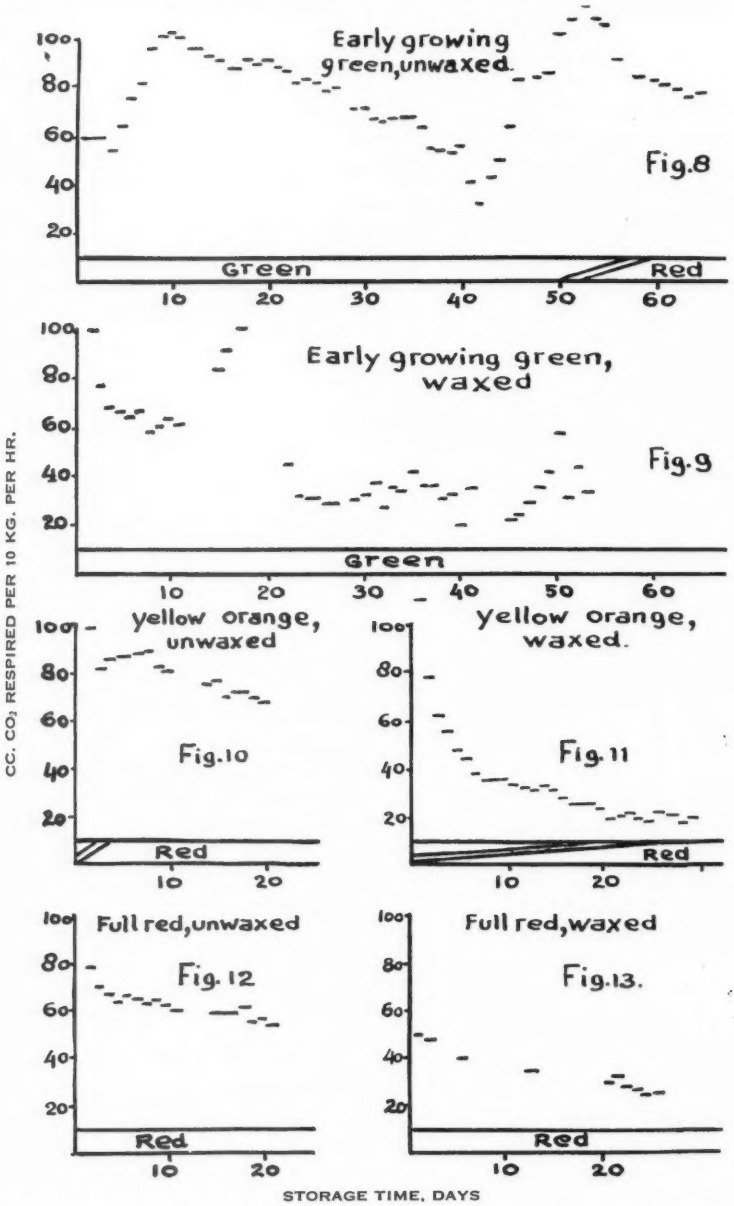
In unwaxed fruits with an initial carbon dioxide content of 7% and oxygen content of 19.3%, the percentage of each increases slightly during storage. During ripening the rising rates of carbon dioxide emission are accompanied by a rising internal carbon dioxide content.

Fruits waxed as soon as picked show a marked increase in carbon dioxide content and a decrease in oxygen content during one week's storage. On removing the wax from the stem scar the carbon dioxide content decreases and the oxygen content increases to such an extent that within 24 hr. from the time of wax removal the internal atmosphere is practically the same as in fruits that had never been subjected to the wax treatment.

This shows beyond doubt that the high "respiration" value observed immediately after wax removal is caused by a liberation of carbon dioxide held in the tissues. Such an early establishment of a new equilibrium indicates a low retentive capacity of the tomato fruit for accumulated carbon dioxide. Measurements of the rate of emission from unwaxed fruits (apparent respiration) thus should give a reasonably accurate indication of the true rate of respiration. Where there have been after-effects of the waxing treatment, i.e., failure to recover their conventional ripening and respiratory behaviour, the previous internal carbon dioxide content rather than the retention of carbon dioxide after wax removal is held to be responsible.

The Effect of Wax Application on Fruits Picked at Various Stages of Maturity

Figs. 9, 5, 11, and 13 are typical of the respiration records obtained on applying a wax seal to the calyx scar of fruits picked and stored at the "growing green" stage (weight, 15 to 25 gm.) and when mature green, yellow orange, and full red, respectively. The progressive changes in the respiration rate of fruits picked at these same maturities and stored without waxing the stem scars are shown in Figs. 8, 1, 10, and 12.



FIGS. 8 TO 13. *Effect of waxing on respiration rate of early growing green, yellow orange, and full red tomato fruits in storage.*

With fruits picked at the mature green and later stages, the general effect of wax application is the same. Beginning at initial rates that depend on the intensity of respiration on the vine at the time of isolation (1, 7, 14, 16), the rate of carbon dioxide output then is lowered progressively because of the modification of the internal atmosphere. The colour changes associated with ripening are seriously inhibited or they may be stopped entirely. In two or three weeks' time, a steady minimal rate of respiration is attained; this is usually of the same order for fruits picked at all stages of maturity from mature green onwards.

The respiration of fruits picked and stored at various growing green stages has been dealt with rather extensively by Walford (16). He regularly found two distinct peaks in the respiration rate if the fruits ripened in storage. He attributed the first of these to an accumulation of sugars at the expense of reserve carbohydrates. As the second peak in respiration rate was associated with the ripening colour change, Walford decided that it was the usual respiratory climacteric.

The respiration records of waxed growing green fruits, as typified in Fig. 9, show differences that distinguish them sharply from those of unwaxed sister fruits (Fig. 8). Waxed growing green fruits have shown consistently higher initial rates of respiration than have the unwaxed individuals, a phenomenon that has never occurred with fruits picked at later stages. In some instances the first peak in the rate of carbon dioxide output of unwaxed fruits (Fig. 9) appears to be abolished entirely by waxing. More often this respiration phenomenon has been postponed and the amount of extra carbon dioxide evolved in it has been diminished because of its shorter duration in time. However, the peak values that are attained in this phase of the records of waxed growing green fruits are frequently quite as high as those shown in the first respiration peak of unwaxed sister fruits.

Until more is known of the concurrent metabolism of waxed fruits of this physiological stage, the peak shown in Fig. 9 is taken to be the initial peak in respiration rate of Fig. 8 as modified by waxing. In some instances waxing has resulted in its complete suppression; more often it has occasioned a postponement of the sharp rise and an abbreviation of the phase of high carbon dioxide production as a whole. Thereafter the respiration rate falls, finally attaining a level approximately the same as that of sealed fruits picked at later stages of maturity.

Tomato fruits picked when only one-quarter grown (25 gm. or less) have never failed to ripen in storage at 12.5° C. so long as the stem scar area was not sealed with wax. When the stem scar area was waxed the fruits have always become subject to one or more of the physiological disorders described below, before there was any trace of ripening coloration.

It has been found (unpublished data) that during the initial rise in respiration rate (Fig. 8) of unwaxed early growing green fruits there is a marked increase in both sugar and acid content and a decrease in protein nitrogen.

At the same time the seeds are enlarging so that as ripening commences the seeds have become full sized and the gelatinous pulp in which they are embedded becomes deliquescent.

The initial increase in respiration is caused evidently by the increasing acid and sugar content. Seed development proceeds as when the fruit is growing on the plant and its carbon requirements may explain the decrease in respiration rate prior to the onset of ripening. At the time that seed development is terminated these small fruits then undergo a typical ripening colour change accompanied by a senescent rise and fall in carbon dioxide emission.

The respiration records of fruits picked and stored unwaxed at the mature green stage with the stems either attached or detached deserve further comment (Fig. 1). The initial rate of carbon dioxide output at 12.5° C. is between 40 and 60 cc. of carbon dioxide per 10 kg. per hr. The rate remains steady or decreases slightly until the onset of the senescent rise. Rising rates of respiration are observed for three or four days before external yellowing of the fruit is visible. The rate continues to rise another two to four days until the fruit is yellow orange with green persisting to some extent at the shoulder. The ascending arm of the tomato fruit's climacteric is then of five to seven days' duration, a period that is considerably shorter than this phase of the respiration record of apples and pears at a temperature of 12° C. (9, 11). The maximal values attained are 50 to 100% higher than those shown before the onset of the senescent rise, values between 70 and 100 cc. being usual. In comparison, the apple shows maximal rates of the same order while those of the pear are considerably higher (9, 11).

After reaching the peak of its climacteric the respiration of the tomato now undergoes little change or declines slowly until the fruit is full red; this requires an additional five to seven days beyond attainment of the peak. Softening of the fruit occurs to a variable extent in its subsequent storage and the accompanying respiratory drift is usually a slow decline (Fig. 1) but occasional complications have occurred in the form of plateaus and secondary peaks. Similar complications have been recorded for the McIntosh apple in storage at 22° C. (12).

Owing to high susceptibility to the common storage moulds in this late senescent phase, the storage life of the unwaxed tomato fruit may be terminated at any point along this phase in which the respiratory rate slowly declines. Fruits that have not been attacked by fungi for as long as 50 days after attainment of the peak of the climacteric have shown values of between 40 and 50 cc. of carbon dioxide with succeeding values the same or slightly lower until fungal invasion did occur.

The final rate of carbon dioxide output observed in senescent tomatoes at the time of fungal attack is frequently of the same order as the values shown immediately before the onset of the climacteric. Krotkov (12) has drawn attention to this equivalence of rate attained in the McIntosh apple. Krotkov suggests that these minimal rates observed before and after the senescent rise indicate a critical metabolic state, the first leading to the deep seated

changes associated with the senescent rise and the second leading to the "pathological" death of the fruit.

The changes in sugar and acid content associated with the ripening of unwaxed tomatoes during storage result in similar concentrations of these constituents at the mature green and full red stages (unpublished data). It is possible that this similarity before and after ripening is responsible in part at least for the observed similarity in respiration rate. It is the author's opinion, however, that one should look elsewhere for the metabolic basis of the senescent rise and increased susceptibility to fungi that terminate the mature green and full red stages, respectively.

Sound mature green tomatoes stored at 12.5° C., and 80 to 85% relative humidity, with stems attached, only become susceptible to storage fungi after the fully ripened condition is reached. This increase in susceptibility to storage rots is believed to be associated with the accompanying pectic changes, the hydrolysis of protopectin to soluble pectin allowing the fungal hyphae ready access to the fruit through intercellular invasion.

Sound mature green tomatoes stored under similar conditions but with stem scars waxed do not show the simple relation between degree of ripening coloration and susceptibility to fungi that exists in unwaxed fruits. It has been observed that waxed fruits become soft before ripening with sufficient frequency to account for most of this lack of correlation. If the basis of increasing susceptibility of fully ripened fruits with stem scars unwaxed is attributable to changes in their cell wall constituents, the same physiological basis of susceptibility to fungi is to be expected in effectively waxed fruits. In the first instance, the cell wall changes accompany the ripening colour change. In waxed fruits the ripening colour change is inhibited while the cell wall changes are not, at least in those instances in which fungi invade waxed fruits not of full ripe colour.

Recapitulating, Walford recorded two general types of respiration for fruits picked and stored when either mature green, yellowing, yellow orange, or full red. The conventional types represented the completion of those phases of the climacteric that had not been completed while the fruit was attached to the plant whereas in the anomalous types the initial rates varied with the maturity at picking and subsequently declined to a rate of 20 to 30 cc. per 10 kg. per hr. with a marked inhibition of further colour change.

In all fruits of these same stages of maturity, stored with the stems attached or with the stems removed but the stem scar unwaxed, records of the conventional type have been found. Waxing the stem scars of such fruits with paraffin has resulted in the consistent appearance of the anomalous or autogas stored types. In all growing green fruits stored with stem scars unwaxed the type previously reported by Walford (16) was obtained. Waxing the stem scars of such fruits resulted in certain modifications of the "initial peak" in carbon dioxide output and physiological breakdown occurred before the appearance of any ripening colour.

In following Walford's technique of applying wax to the stem scar of all fruits studied, Singh and Mathur (15) record examples of the "auto-gas stored" or anomalous type among fruits picked green. All fruits picked at later stages of ripeness showed distinct peaks in carbon dioxide output that appear to be quite unrelated to any accompanying colour changes. If the internal ripeness of their fruits is indicated accurately by skin colour under their conditions, these records must be interpreted as postclimacteric phases of unsealed or partially sealed fruits. A repetition of their observations on such fruits, sealed and unsealed, should prove of interest.

It has been shown above that the anomalous type owes its characteristics to the presence of the wax seal since removal of the wax causes the immediate disappearance of anomalous characteristics while reapplication of wax causes them to appear once more. The initial values of the rate of carbon dioxide emission immediately after wax application and wax removal and the demonstrated changes in the internal atmosphere that result from wax application and wax removal demonstrates that auto-gas storage is the physiological basis of the anomalous and intermediate storage types.

The Functional Disorders of the Waxed Tomato in Storage

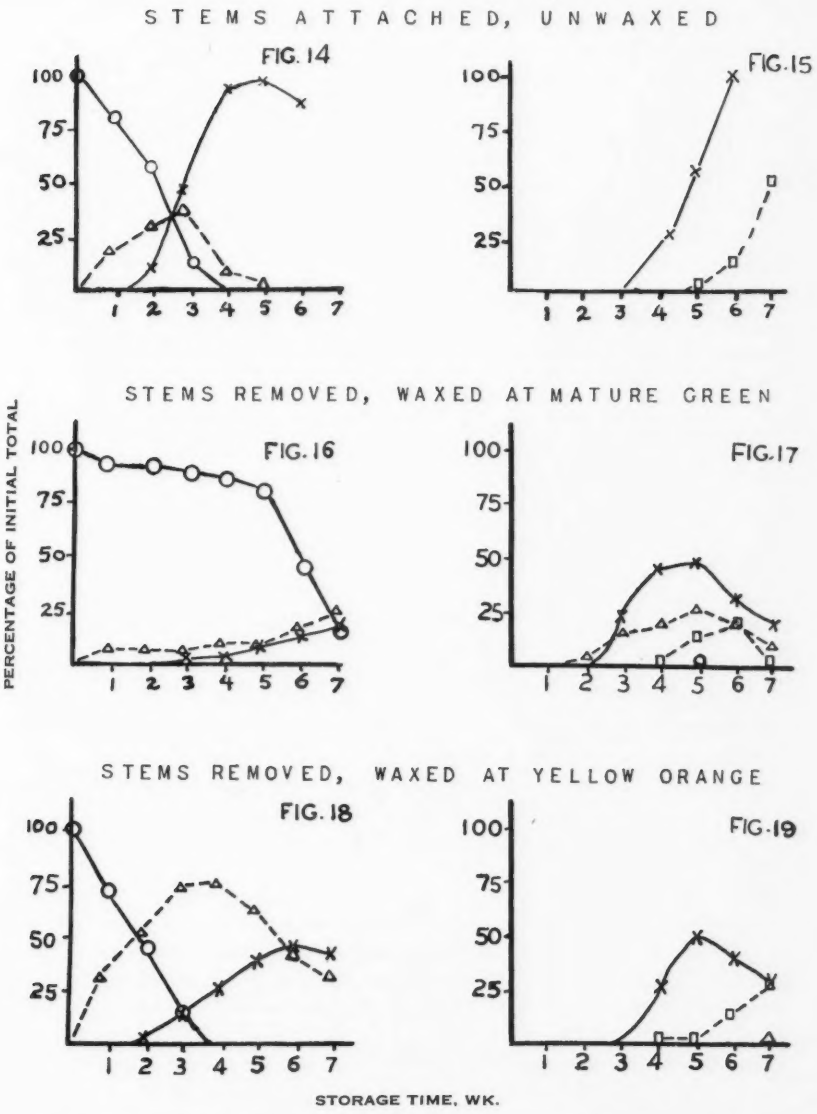
The unusual durability in storage of certain tomatoes of the anomalous or sealed type has made this investigation of considerable interest from a commercial standpoint. Assuredly any cultural or storage practices that appear to have an effect on the mean storage life of fruits are worthy of careful study.

Waxing the stem scar has been recommended elsewhere as a routine commercial practice (4). The experience of the author is that this recommendation is as yet unwarranted. Extension of the storage life of tomatoes by this means appears promising however since the rates of transpiration, respiration, and ripening are greatly diminished. However, at no time has this technique resulted in an increase in the mean storage life in extensive bulk storage trials; this is attributed to undesirable accompaniments which appeared principally in the form of physiological disorders.

The application of wax results first of all in wide differences in ripening rate. This is attributed to a magnification of unavoidable differences in the maturity of the fruits at picking and in the degree of sealing effected by waxing the stem scars. Waxed tomatoes also show great differences in the rate at which different parts of the same fruit attain ripe colour. It follows that some method of overcoming this induced heterogeneity (5) should be found before attempting the waxing technique on a commercial scale.

Several physiological disorders, previously unrecorded, have been observed as detrimental accompaniments of the waxing technique. A description of these disorders is included below. The numbers of fruits that may be expected to become affected with them also are indicated for different waxing treatments (Figs. 14 to 19).

Premature softening is characterized, as its name implies, by a serious loss of firmness before the initiation or during early stages of the ripening colour



FIGS. 14, 16, AND 18. *Effect of waxing on ripening behaviour of tomato fruits at 12.5° C. Stage of maturity:* ○ = *mature green;* △ = *ripening;* × = *ripe.*

FIGS. 15, 17, AND 19. *Effect of waxing on the development of disorders. Type of disorder:* ○ = *surface browning;* △ = *surface pitting;* × = *premature or senescent softening;* □ = *fungal infection.*

change. These soft fruits may acquire a dark, sodden appearance over the locules and at the blossom end. On removal to higher temperatures or on removal of the wax seal, they fail to ripen properly. This disorder is associated with a marked susceptibility to the common storage moulds.

Surface pitting first becomes evident as small white pockets in the skin of the preclimacteric fruit, usually near the blossom end. At later stages more and more of the fruit surface shows this disorder with the small sunken areas tending to merge. Ripening has not been observed in fruits showing these symptoms on removal of the wax or on storing at 70° C. The use of oiled wraps in a number of bulk storage trials has had no effect whatsoever on the number of fruits that become pitted. Attempts to isolate causal organisms from the pits have failed but pitted fruits are noticeably susceptible to fungal attack particularly in the sunken areas. This disorder evidently involves a preliminary physiological breakdown of the skin cells followed by their desiccation and collapse.

Surface pocketing involves lesions of larger area than does pitting. The lesions are deeper and are not necessarily associated with a whitish appearance. In the particular storage experiment recorded below this disorder is included under "surface pitting".

Surface browning appears as superficial brown blotches or smears at or near the blossom end, gradually spreading upwards. This disorder is not related to a collapse of the skin tissue nor is it associated with any noticeable increase in susceptibility to fungus. On removal of the wax seal the result has been a completion of the ripening colour change in all parts of the fruit except at regions showing the injury. Here the brown colour of the skin persists while the underlying wall tissue ripens to a variable extent. The respiratory drift of such a fruit has been followed before and after wax removal, and a month after wax removal when the stem scar has been once more sealed with wax. The respiration record obtained during this manipulation of wax has corresponded in every detail with that shown in Fig. 7 for normal fruits, the rise in respiration rate after wax removal being associated with the ripening colour change.

Fruits picked at any stage of maturity and stored at 12.5° C. with stem scars unwaxed are not subject to the above disorders. Fruits stored at the mature green stage, with the stem scars waxed, may show any of them. However, if fruits are allowed to remain on the plant until yellow orange or full red and then are stored with stem scars waxed, pitting and browning do not occur to any extent.

The above observations suggested that a feasible method of controlling pitting, browning, and unequal ripening rates might be to postpone wax application until the fruits attain considerable ripening coloration in storage. Accordingly an experiment was conducted using different varieties of tomatoes to ascertain if such a method of control was feasible.

In this experiment the fruits were divided into three uniform lots. The first lot was stored with the stems attached throughout, thus serving as a

check. The stems were removed from the second lot and before being stored the scars were covered carefully with paraffin. The third lot was stored with the stems attached until the fruits coloured to yellow orange, thereupon their stems were removed and the stem scars were waxed carefully.

Figs. 14 to 19 give the results obtained with the Grand Rapids variety, using fruits of a 1940 early spring greenhouse population. The general picture for this variety as regards ripening rate and incidence of fungal and physiological disorders applies equally to the other varieties and hybrids tested simultaneously. In the experiment under discussion fruits were not discarded until attacked by fungi. Thus, owing to the removal of such fruits, Fig. 17 shows a decline in the numbers of fruits affected with presenescent softening, pitting, and browning. The actual wastage is not quite as great as the data indicates since all values for wastage represent "total" counts for each disorder and some fruits showed more than a single form of breakdown.

Figs. 14, 16, and 18 were obtained by counting the numbers of fruits of each ripening class after the storage periods indicated and by expressing these counts as percentage of the initial total. After four weeks' storage, practically all unwaxed fruits (Fig. 14) were either ripening or ripe while 85% of the fruits waxed initially were still mature green (Fig. 16). At five weeks all unwaxed fruits were full red while the "waxed initially" lot showed only 10% either orange or red.

Figs. 16 and 17 show the extreme heterogeneity to be expected after five to seven weeks' storage within a population of fruits picked mature green and stored at 12.5° C. with stem scars sealed carefully with paraffin. Fruits of all degrees of ripeness from mature green to full red are seen to be present in large numbers and, in each ripening class, storage disorders are serious.

The application of wax to the stem scar when the yellow orange stage is attained in storage results of course in a marked increase in the number of fruits at this stage of ripening. The wax seal serves to maintain the fruit at the stage at which the wax is applied by inhibiting further ripening coloration. Mention was made earlier of the heterogeneity of fruits waxed at the mature green stage. This does not apply to the same extent to fruits allowed to ripen partially before wax is applied but the greatest homogeneity in this respect is still found in lots stored with the stems attached throughout. The first evidence of wastage among fruits of the check lot was senescent softening of the full red fruits with negligible amounts of wastage of other kinds up to six weeks' storage.

The original purpose of this bulk storage experiment was to test the efficacy of delayed waxing in controlling pitting and browning and heterogeneity within the population with respect to ripening rate. Fig. 19 shows that delayed waxing will eliminate these undesirable accompaniments of earlier wax application. But unfortunately, delayed waxing served to augment the numbers of fruits becoming soft prematurely and the severity of this effect discounts the advantages cited above for such a practice.

The waxing of the stem scar has not resulted in any control whatsoever of fungal attacks at either the stem scar or other areas. The greatest freedom from fungal attack is found consistently in unwaxed fruits stored with the stems attached. Application of a wax cover has even been observed to favour the growth of moulds in many instances. As suggested by Claypool (6) the effect is evidently caused by the higher relative humidity existing immediately under the wax.

In view of the undesirable effects of waxing cited above, it is considered inadvisable at present to recommend wax application to the stem scars as a commercial practice. A treatment of the stem scar area that results in an ideal restriction of carbon dioxide emission and in a complete restriction of water loss will be sought in further experiments. In the meantime Canadian grown tomatoes of high quality may be held satisfactorily for five weeks by storing at the mature green stage with stems attached, at a temperature of 10 to 12.5° C., and at a relative humidity of 80 to 85%.

Acknowledgments

The author wishes to express his appreciation to Professor G. H. Duff and Dr. J. H. L. Truscott for their advice and criticism during the course of this investigation. The author is indebted to Mr. W. R. F. Grierson-Jackson who conducted the internal atmosphere analyses and to Mr. E. R. Waygood and Mr. R. M. Ferguson who assisted with the respiration and bulk storage studies. Financial support for this investigation was provided by the Research Fund of the Ontario Agricultural College.

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LA DIAPAUSE CHEZ LES TENTHRÈDES

PARTIE II[1]

PAR A. R. GOBEIL

Influence d'immersions sur la rupture de la diapause

TECHNIQUE ET CONSIDÉRATIONS GÉNÉRALES

Dans la première partie du présent travail nous avons vu que plusieurs auteurs, entre autres, Townsend (114) et Babcock (4), ont insisté sur l'influence prépondérante de l'immersion préalable des cocons dans l'eau pour amener la rupture de la diapause. Dans leur milieu naturel, les cocons de la mouche à scie européenne de l'épinette, de même que ceux de la mouche à scie du mélèze sont fréquemment en contact avec l'eau. De plus cette eau a un pH généralement acide et l'on peut se demander avec raison si cette condition n'influence pas la mortalité et le développement de la larve dans le cocon. C'est ce que j'ai cherché à vérifier dans une série d'expériences dans lesquelles j'ai utilisé, en plus de l'eau distillée, des solutions acides à des pH de 5 et de 3. Deux acides furent employés pour obtenir ces pH: un inorganique, l'acide sulfurique (H_2SO_4), et l'autre organique, le glycocolle (NH_2CH_2COOH).

Townsend (114) dans ses recherches sur la pyrale du pommier en était arrivé à la conclusion que des immersions répétées et de courte durée sont plus efficaces pour provoquer la reprise du métabolisme normal après l'hibernation que des immersions longues et moins fréquentes. Les deux sortes d'immersions furent donc mises à l'essai. Pour *D. polytomun* Htg., j'ai fait dans certains cas (Série *a*) trois immersions d'une heure chacune, les lundi, mercredi, et vendredi; alors que dans d'autres cas (Série *b*) je ne fis qu'une immersion de deux heures consécutives le mercredi. Dans le cas de *P. erichsoni* Htg. qui vit généralement dans un milieu plus humide que *D. polytomum*, j'ai procédé aux immersions suivantes: deux heures consécutives le mercredi (Série *b*); quatre heures consécutives le mercredi (Série *c*); deux heures consécutives les lundi, mercredi, et vendredi (Série *d*).

Les individus subissant des immersions dans l'eau distillée furent divisés en trois groupes: 1. Immersions au cours de l'hibernation seulement (34°F.); 2. Immersions durant la nymphose seulement (75°F.); 3. Immersions durant l'hibernation et la nymphose. Dans le cas des solutions acides, à moins d'indication contraire, les immersions ont toujours eu lieu à 34° et 75°F.

Notre façon de procéder dans toutes ces expériences d'immersions forcées consistait à submerger les tubes de verre contenant les cocons dans des

[1] *La Partie I a paru dans le numéro de novembre.*

bassins d'eau, *dont la température était celle du milieu.* Après l'opération, ces tubes étaient simplement secoués afin d'enlever toute l'eau possible. Le grand nombre d'expériences que j'avais en marche sur différents genres d'immersions (182 lots de 100 cocons chacun) permettait difficilement un traitement plus minutieux. J'ai vite constaté, cependant, surtout lors des immersions en incubateur, que cette façon de procéder était inadéquate. Le cocon n'étant pas asséché, il restait toujours sur la surface une légère couche de liquide, de sorte qu'en réalité la durée de l'immersion se trouvait être plus longue que la période spécifiée. De plus, les fortes secousses imprimées aux tubes de verre pour enlever l'eau endommageaient les cocons, surtout ceux de *P. erichsoni* dont la paroi est beaucoup moins rigide. Comme conséquence, le fort taux de mortalité constaté dans plusieurs cas pouvait être aussi bien attribué à la manipulation qu'à l'immersion elle-même.

Pour les individus ayant passé 8 et 12 semaines en hibernation, j'ai donc modifié ma technique. Après chaque immersion, les cocons furent asséchés entre deux buvards réduisant ainsi au minimum la mortalité due à la manipulation.

Vitalité de Diprion polytomum dans l'eau

Avant de faire le choix des différentes catégories d'immersion mentionnées plus haut, j'ai cherché au préalable à déterminer la résistance de *D. polytomum* dans l'eau à 34° et 75°F.

Dans nos expériences d'immersions à 34°F., 1500 cocons furent placés dans un bocal qui fut ensuite rempli d'eau. A tous les jours, 50 cocons étaient ouverts et l'état des individus noté. Nous avons procédé de la même façon pour les immersions effectuées à 75°F., mais comme tous nos cocons étaient morts après quatre jours d'immersion, il nous a fallu recommencer l'expérience et procéder à nos examens à des intervalles variant de 5 à 24 heures entre chacun.

Lorsque le cocon a passé plusieurs heures dans l'eau, la larve devient comme asphyxiée et il est souvent impossible au moyen d'un examen sommaire de déterminer si elle est vivante ou morte. Pour être sûr de ne pas faire d'erreur il faut, après que les larves ont été extraites de leur cocon, laisser tous les individus douteux dans l'incubateur au moins une journée avant de les classer définitivement. Après un stage de 24 heures en incubateur, si on presse légèrement la larve asphyxiée mais vivante, on peut, pour le moins, voir remuer les pièces buccales. D'autre part, nous avons noté qu'en incubateur (75°F. et 85% R. H.*), la larve extraite de son cocon peut vivre au moins neuf jours.

Il faut un stage d'au moins 26 jours dans l'eau à 34°F. pour obtenir 100% de mortalité, alors qu'à une température de 75°F. les larves ne résistent que huit jours au maximum (Tableau XI). En conséquence, il est raisonnable de penser qu'en juillet et en août, un stage de trois ou quatre jours dans un milieu saturé d'eau sera fatal à plusieurs larves. Par contre, lors de la fonte

* *R.H. = humidité relative.*

TABLEAU XI

RÉSISTANCE DE *Diprion polytomum* DANS L'EAU

Immersions à 34°F.				Immersions à 75°F.	
Nombre de jours	Mortalité, %	Nombre de jours	Mortalité, %	Nombre d'heures	Mortalité, %
1	5.6	13	62.1	5	2.0
2	8.6	14	63.4	10	4.0
3	11.8	15	66.0	15	7.0
4	15.0	16	69.8	20	7.5
5	25.5	17	67.0	25	12.8
6	31.0	18	72.0	30	20.0
7	37.8	19	74.0	50	24.0
8	41.7	20	78.5	55	30.0
9	45.0	21	82.0	72	85.5
10	52.7	22	86.0	96	96.0
11	57.3	23	92.0	108	100
12	60.6	26	100		

des neiges certains individus pourraient vivre près d'un mois, même s'ils sont complètement submergés.

Constitution de l'humus et acidité des sols forestiers

J'aurais voulu préparer des solutions d'humus au moyen de composés chimiques définis mais, suivant Waksman (119), la chose est impossible, l'humus n'étant pas un composé pouvant être représenté par une formule bien que plusieurs aient été mises en évidence par différents auteurs. D'ailleurs le mot humus n'est pas toujours employé pour désigner les mêmes substances organiques ou préparations. Certains appliquent ce terme à toute la matière organique du sol, alors que d'autres désignent par ce mot seulement cette partie de la matière organique qui est facilement oxydée par certains réactifs.

Quant aux composés chimiques constituant cette matière foncée du sol, un nombre considérable de formules ont été préparées et il semble y avoir eu beaucoup de confusion sur la classification et composition de ce qu'on a appelé, l'humus acide, l'acide humique, etc. Même maintenant, les opinions sont encore partagées sur la nature chimique de l'humus et les groupes qui le constituent. Plusieurs considèrent encore les acides humiques comme les plus importants constituants de l'humus et certains chimistes sont encore convaincus que les acides humiques obtenus en traitant des carbohydrates avec des acides minéraux sont identiques à ceux présents dans les résidus de plantes en décomposition que l'on trouve dans le sol. Après avoir passé en revue la série de formules émises pour représenter l'humus ou l'acide humique, Waksman remarque que le plus important élément de l'humus est l'azote mais que ce corps est généralement omis des différentes formules. Il en conclut que la nature chimique de l'humus n'a pas encore été établie et qu'on ne peut représenter ce complexe par une formule de constituants qui ne sont pas des composés chimiques spécifiques.

Suivant le même auteur, l'humus représente un complexe hétérogène constitué de plusieurs composés d'origine végétale, animale, microbique et de leurs produits de décomposition et de transformation. Bien qu'une foule de composés chimiques peuvent être isolés de l'humus, ce dernier est caractérisé par certains groupes généraux de constituants, variant qualitativement et quantitativement suivant la nature et l'origine de l'humus. On est, par conséquent, justifié de parler de l'humus, non pas comme un complexe chimique, mais comme un état de la matière. De même que les plantes et les substances animales peuvent être sujettes à des analyses chimiques et séparées en plusieurs groupes généraux de composés chimiques possédant des propriétés semblables, de même l'humus peut aussi être divisé en groupes de complexes spécifiques. L'humus originant de la décomposition de matières végétales et animales, la nature des substances qui le constituent change donc continuellement à mesure que progresse la décomposition.

Vu l'impossibilité de préparer, au moyen de composés chimiques définis, des solutions acides qui auraient pu représenter différents types d'humus, il fut décidé d'obtenir les pH désirés au moyen de deux acides: l'acide sulfurique et le glycocolle. Dans plusieurs sols, les composés nitrogénés constituent à peu près un tiers de l'humus. Ces composés donnent par hydrolyse des acides aminés et, suivant plusieurs auteurs, la formation de l'humus ne serait que le résultat de la condensation de carbohydrates avec des acides aminés, dont le glycocolle.

A quelques exceptions près, nos types d'humus forestiers dans Québec sont toujours acides, le pH pouvant être aussi bas que 3.0. Suivant le Prof. L. Z. Rousseau, de l'École forestière de l'Université Laval, l'humus brut à structure laminée, appelé aussi mor ou duff, a un pH généralement inférieur à 4.5 avec une moyenne de 3.5 à 4.0. L'humus doux ou mull, à structure granuleuse, difficile à distinguer du sol minéral et dont la couche est généralement minime comparée à l'humus brut, a un pH variant de 4.5 à 7.0 avec une moyenne de 5.6 à 6.0.

Préparation de solutions à pH 5 et pH 3

Les solutions que nous avons utilisées furent préparées par le Dr L. Gravel, chimiste, et furent vérifiées au moyen de l'électrode de verre.

Les solutions d'acide sulfurique furent obtenues en diluant dans un gallon d'eau distillée 45 cc. d'acide sulfurique à 0.101 *N* pour les solutions à pH 3 et 45 cc. à 0.00101 *N* pour les solutions d'un pH 5.

Il est impossible d'avoir des solutions de glycocolle à pH 3 (117). Pour ce composé les solutions furent donc tamponnées au moyen de l'acide acétique.

Les solutions d'acide sulfurique n'étant pas tamponnées leur pH varie plus facilement sous l'influence d'agents chimiques augmentant ou diminuant le nombre d'ions H^+. Ces solutions furent donc vérifiées trois fois par semaine et renouvelées chaque fois que le pH était supérieur à 3.

Mortalité durant l'hibernation

Diprion polytomum

Les chiffres présentés au Tableau XII et leur analyse au Tableau XIII nous permettent de tirer les conclusions suivantes sur l'effet d'immersions dans différentes sortes de solutions aqueuses au cours de l'hibernation:

TABLEAU XII

Diprion polytomum, influence d'immersions sur le pourcentage de mortalité durant l'hibernation

Hibernation en jours	Blocs	H_2O		H_2SO_4 à pH 3		NH_2CH_2COOH à pH 3	
		2 hres	3 hres	2 hres	3 hres	2 hres	3 hres
42	I	16	20	20	20	24	16
	II	16	16	24	24	20	20
56	I	0	16	12	36	20	40
	II	20	24	20	40	36	48
84	I	20	28	20	16	28	36
	II	20	32	16	24	32	40
112	I	28	28	12	16	24	24
	II	20	32	16	20	20	28
Moyenne		17.5	24.5	17.5	24.5	25.5	31.5

TABLEAU XIII

Analyse de variance sur la mortalité durant l'hibernation

(Voir Tableau XII)

Sources de variation	*D.L.*	Variance
Blocs	1	161.33
Traitements	23	134.43**
Jours	3	114.33**
Solutions	2	300.00**
Immersions	1	533.33**
Jours × Sol.	6	176.66**
Jours × Imm.	3	141.56**
Sol. × Imm.	2	1.38
Jours × Sol. × Imm.	6	20.90
Erreur	23	19.42
Total	47	Erreur standard = 4.41

Différences significatives: Jours = 3.73
Solutions = 3.23
Immersions = 2.64
Jours × Sol. = 6.46
Jours × Imm. = 5.27

** *Dépasse "erreur" variance, niveau significatif de 1%.*

1. La différence significative que l'on constate dans le pourcentage de mortalité après différentes périodes d'hibernation s'explique par les chiffres exceptionnellement élevés pour six et huit semaines d'hibernation chez les individus ayant subi des immersions dans le glycocolle. Normalement, cependant, la durée de l'hibernation n'affecte pas le pourcentage de mortalité, même si, au cours de celle-ci, les cocons subissent des immersions de deux ou trois heures par semaine dans différentes solutions aqueuses.

2. Après hibernation à 34°F., sans immersion (Tableau I*), nous avions obtenu 21.5% de mortalité. Lorsque l'hibernation comporte des immersions de deux heures par semaine dans l'eau ou dans une solution d'acide sulfurique à pH 3, nous avons exactement 21.0% (Tableau XII). C'est dire que celles-ci ne sont aucunement défavorables à la larve. D'autre part, les immersions dans le glycocolle, avec 25.1% de mortalité, sont décidément incompatibles aux individus en hibernation.

3. Un séjour de deux heures par semaine dans des solutions aqueuses (20.2% de mortalité) n'affecte pas la larve, mais si la durée des immersions est portée à trois heures, nous créons alors un milieu défavorable puisque nous avons 26.8% de mortalité et que la différence significative n'est que de 2.6%.

4. Lorsque le stage à 34°F., est relativement court, 42 jours, la nature de la solution aqueuse employée de même que la durée des immersions n'affectent pas le pourcentage de mortalité. Mais lorsque la période d'hibernation est supérieure à six semaines, les immersions dans les solutions de glycocolle, que ce soit de deux ou trois heures, de même que toutes les immersions de trois heures en général, que ce soit dans l'eau, l'acide sulfurique ou le glycocolle, sont fatales à une proportion beaucoup plus considérable des sujets.

Pristiphora erichsoni

Le résultat de nos observations nous porte à croire qu'à l'encontre de ce qui a été constaté pour *D. polytomum*, la nature de la solution aqueuse n'influence pas la mortalité chez la mouche à scie du mélèze. Mais vu le petit nombre d'individus prélevé sur chaque lot et examiné à la fin de l'hibernation, il ne fut pas possible de faire une analyse de variance aussi complète que celle présentée au Tableau XIII pour *D. polytomum*. Toutefois pour les simples immersions dans l'eau, vu que durant l'hibernation quatre lots reçurent les mêmes traitements, j'ai pu, en groupant mes résultats tels que donnés au Tableau XIV, en faire l'analyse statistique et déterminer l'influence des immersions de différentes durées pour différentes périodes d'hibernation.

Bien que les variations dans le pourcentage de mortalité après différentes périodes d'hibernation soient assez considérables (Tableau XIV), l'analyse de variance du Tableau XV nous prouve que ces différences ne sont pas suffisantes pour nous donner raison d'attacher une importance quelconque aux variations enregistrées. Il en est de même pour l'interaction de la période d'hibernation sur la durée des immersions.

**Dans la Partie I.*

TABLEAU XIV

Pristiphora erichsoni, INFLUENCE D'IMMERSIONS DANS L'EAU SUR LE POURCENTAGE DE MORTALITÉ DURANT L'HIBERNATION

Traitements	Répétitions	Hibernation en jours			
		42	56	84	112
Aucune immersion	1	12	0	20	10
	2	4	50	10	20
	3	24	10	10	20
	4	16	20	20	20
2 hres d'immersion	1	20	30	60	30
	2	24	50	50	20
	3	24	10	20	20
	4	24	0	30	30
4 hres d'immersion	1	28	20	40	10
	2	28	70	30	40
	3	28	0	30	40
	4	24	20	20	10
6 hres d'immersion	1	0	50	50	40
	2	8	40	60	60
	3	32	60	40	20
	4	24	70	40	10
Moyenne		20	31.2	33.1	24.4

TABLEAU XV

ANALYSE DE VARIANCE SUR LA MORTALITÉ DE *Pristiphora erichsoni* DURANT L'HIBERNATION

(Voir Tableau XIV)

Sources de variation	*D.L.*	Variance
Répétitions	3	473.42
Traitements	15	535.58**
Jours	3	593.75
Immersions	3	1122.92**
Jours × Imm.	9	320.42
Erreur	45	212.26
Total	63	Erreur standard = 14.569

** *Dépasse "erreur" variance, niveau significatif de 1%.*

Par contre les variations associées aux immersions sont très prononcées et nous avons une probabilité de beaucoup inférieure à 1%. Pour être significatives, les différences entre les moyennes de mortalité doivent avoir un minimum de:

$$\left(\frac{14.72\sqrt{2}}{\sqrt{16}}\right) 1.96 = 10.$$

Une simple inspection du Tableau XIV nous permet d'affirmer que *Pristiphora erichsoni* peut aussi bien supporter quatre heures que deux heures d'immersions continues par semaine. Mais il n'en est pas de même pour trois semaines de deux heures chacune par semaine, alors que le pourcentage de mortalité monte à 37%. De plus, il semble que la larve s'accommode beaucoup mieux d'une hibernation sans contact avec l'eau (16.6% de mortalité) qu'aux hibernations avec immersions, même si elles ne sont que de deux heures par semaine.

Ces résultats, toutefois, ne sont pas très concluants, parce que, comme je l'ai déjà fait remarquer, le cocon de la mouche à scie du mélèze est très flexible, et dans le cas des lots ayant subi des immersions, il est probable qu'une bonne partie de la mortalité puisse être attribuée à la manipulation. C'est ce qui expliquerait qu'entre quatre et six heures d'immersion nous ayons une différence d'environ 10% de mortalité; le dernier traitement ayant nécessité trois manipulations contre une seulement lorsqu'il s'est agit de quatre heures consécutives d'immersion. D'ailleurs si l'on considère que *P. erichsoni* vit normalement dans un milieu plus humide que *D. polytomum*, il serait surprenant que *Pristiphora* soit affecté par de courtes immersions dans l'eau alors que *Diprion* ne l'est pas.

Mortalité durant la nymphose

La grande majorité des larves de *Pristiphora erichsoni* ayant subi des immersions dans des solutions aqueuses étaient mortes après 60 jours en incubateur. Même lorsque les cocons furent asséchés en les secouant légèrement entre deux buvards, comme ce fut le cas pour certains lots ayant passé 12 semaines d'hibernation, le pourcentage de larves vivantes à la fin des expériences fut encore inférieur à 3%. Ces résultats ne peuvent pas être attribués aux conditions de milieu, mais plutôt à la manipulation. La technique employée, immersion des tubes suivie de fortes secousses pour enlever l'eau, s'est avérée tout à fait défectueuse pour la mouche à scie du mélèze. Heureusement, il n'en fut pas de même pour *Diprion* et les résultats présentés aux Tableaux XVI, XVIII, et XX nous ont permis des constatations intéressantes.

Immersions dans l'eau

L'analyse statistique du Tableau XVII démontre que:

1. Lorsque le cocon est en contact avec l'eau, le taux de mortalité en incubateur est nettement plus élevé après 56 jours d'hibernation (52.6%) qu'après 42 jours (37.3%). D'autre part, les différences observées lorsque l'hibernation varie de 56 à 116 jours sont négligeables. Dans les conditions naturelles, la diapause, lorsque diapause il y a, est généralement supérieure à

huit semaines. Au printemps, lorsque la température devient favorable à la réactivation, la résistance de la larve dont le cocon est souvent en contact avec l'eau ne serait donc pas influencée par la durée de l'hibernation.

TABLEAU XVI

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS L'EAU SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Blocs	Jours à 34°F.	Imm. à 34°F.		Imm. à 75°F.		Imm. à 34° et 75°F.	
		2 hres	3 hres	2 hres	3 hres	2 hres	3 hres
I	42	51.2	30.1	51.6	46.1	38.8	26.8
	56	34.7	34.0	55.8	52.6	22.8	100
	84	39.7	49.4	59.2	43.2	42.7	96.9
	112	45.2	45.7	50.5	46.3	55.7	39.5
II	42	36.6	30.1	41.9	43.5	25.0	26.2
	56	37.0	67.6	45.5	48.2	32.9	100
	84	40.2	44.2	59.7	42.3	41.4	90.7
	112	57.7	51.5	42.8	43.3	52.6	44.0
Moyenne		42.8	44.1	50.9	45.6	39.0	65.5

Note: Nymphose à 75°F. et 85% R.H.

TABLEAU XVII

ANALYSE DE VARIANCE SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau XVI)

Sources de variation	*D.L.*	Variance
Blocs	1	3.85
Traitements	23	591.99**
Jours	3	690.66**
Immersions	2	312.12**
Heures	1	682.52**
Jours × Imm.	6	349.62**
Jours × Hres	3	887.66**
Imm. × Hres	2	1146.06**
Jours × Imm. × Hres	6	538.27**
Erreur	23	48.15
Total	47	Erreur standard = 6.94

Différences significatives: Jours = 5.85
Immersions = 5.08
Heures = 4.13
Jours × Imm. = 10.16
Jours × Hres = 8.28
Imm. × Hres = 7.18

** *Dépasse "erreur" variance, niveau significatif de 1%.*

TABLEAU XVIII

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Blocs	Jours à 34°F.	H_2SO_4		NH_2CH_2COOH	
		pH 5	pH 3	pH 5	pH 3
I	42	17.8	59.4	76.5	47.7
	56	37.2	30.3	35.7	63.5
	84	54.2	32.6	54.6	75.5
	112	47.4	47.1	60.6	74.0
II	42	21.5	34.6	31.5	48.0
	56	33.7	27.1	30.6	47.8
	84	48.4	36.9	55.7	93.8
	112	48.5	35.4	63.0	67.0
Moyenne		38.6	37.9	51.0	64.7

Note: Immersions à 34° et 75°F., deux heures consécutives chaque mercredi.

2. Il n'est pas douteux que le contact de l'eau durant l'hibernation et la nymphose entraîne la mort d'un plus grand nombre d'individus (52%) que si le contact ne se fait que durant l'hibernation (43%). Il semble de plus évident que ce sont les immersions durant la nymphose qui sont particulièrement défavorables, la mortalité étant presque aussi élevée (48%) si les immersions n'ont eu lieu qu'à 75°F., que si elles sont produites à 34° et 75°F. Par contre, l'insecte paraît mieux résister au contact de l'eau au cours de l'hibernation que durant la nymphose et, comme question de fait, les immersions de deux et trois heures durant l'hibernation n'affectent en rien le taux de mortalité durant la nymphose. En effet, lorsque la larve n'a eu aucun contact avec l'eau au cours de l'hibernation et de la nymphose, nous n'avons eu que 40% de mortalité après 60 jours d'incubation à 75° et 85% R.H. Ceci ne représente que 3% de moins que lorsqu'il y a immersion à 34°F. et cette légère différence n'a rien de significatif.

3. Si les immersions n'ont lieu que durant l'hibernation ou la nymphose, la larve supporte également bien les immersions de deux et de trois heures par semaine. Mais, lorsque le cocon subit le contact de l'eau au cours de ces deux phases vitales, la larve offre alors moins de résistance à trois immersions d'une heure chacune par semaine qu'à une seule immersion de deux heures consécutives.

Immersions dans des solutions acides

Toutes ces immersions eurent lieu tant en incubateur qu'à 34°F. sauf quelques exceptions qui sont indiquées plus loin.

Les faits saillants qui ressortent des expériences comportant des immersions dans des solutions acides sont les suivants:

1. Comme pour les immersions dans l'eau, le taux de mortalité est en relation étroite avec la durée du stage à 34°F., et dans le cas présent il est plus élevé après 12 et 16 semaines qu'après six et huit semaines d'hibernation.

2. Si l'on compare les résultats des Tableaux XVI et XVIII, on constate d'une part que *D. Polytomum* réagit aussi bien aux immersions dans les solutions d'acide sulfurique (38% de mortalité) qu'aux immersions d'eau pure (39%). D'autre part, les immersions dans les solutions de glycocolle provoquent un plus fort pourcentage de mortalité (58%), et la différence avec l'acide sulfurique est très marquée (Tableau XIX). D'après le Dr J. Ls. Tremblay, professeur de biologie à Laval, le glycocolle serait en l'occurence toxique.

TABLEAU XIX

ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau XVIII)

Sources de variation	*D.L.*	Variance
Blocs	1	256.50
Traitements	15	529.70**
Jours	3	682.18**
Acides	1	3069.36**
pH	1	336.70
Jrs × Ac.	3	75.77
Jrs × pH	3	32.64
Ac. × pH	1	408.98
Jrs × Ac. × pH	3	586.23**
Erreur	15	100.56
Total	31	Erreur standard = 10.03

Différences significatives: Jours = 10.68
Acides = 7.56

** *Dépasse "erreur" variance, niveau significatif de 1%.*

3. L'analyse de variance des Tableaux XIX et XXI démontre que par lui-même le pH n'a aucun effet mortel sur l'individu dans le cocon. La différence significative de 14.7% de mortalité que l'on note entre les immersions dans les solutions de glycocolle à pH 5 et pH 3 ne peut pas être attribuée au pH, mais bien à la concentration d'une substance qui est contraire à la larve.

4. Aucune des interactions jours sur acides et acides sur pH ne diminue de façon sensible la vitalité des individus.

5. Après deux heures consécutives d'immersion hebdomadaire dans une solution d'acide sulfurique à pH 3, le taux de mortalité est de 38% (Tableau XX). Si les immersions sont portées à trois, d'une heure chacune par semaine, la proportion des larves mortes atteint 76%. Dans les solutions de

glycocolle, la différence de mortalité est aussi très prononcée, soit de 64.7 et 93.5%. D'après notre analyse du Tableau XXI, une différence supérieure à:

$$\frac{16.07}{\sqrt{8}} \times 1.414 \times 2.01 = 16.2$$

est significative. Comme pour les immersions dans l'eau, on peut donc conclure que l'augmentation dans le taux de mortalité est en rapport direct avec la durée de l'immersion.

TABLEAU XX

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Blocs	Jours à 34°F.	H_2SO_4					NH_2CH_2COOH		
		pH 5	pH 3				pH 5	pH 3	
		2 hres	2 hres	3 hres	2 hres†	2 hres‡	2 hres	2 hres	3 hres
I	42	17.8	59.4	89.7	16.4	17.0	76.5	47.7	100
	56	37.2	30.3	88.6	27.8	71.5	35.7	63.5	100
	84	54.2	32.6	71.7	35.1	57.2	54.6	75.5	100
	112	47.4	47.1	61.5	43.2	49.4	60.6	74.0	72.4
II	42	21.5	34.6	85.5	16.0	13.6	31.5	48.0	100
	56	36.7	27.1	89.4	34.4	72.5	30.6	47.8	100
	84	48.4	36.9	63.5	48.4	54.5	55.7	93.8	100
	112	48.5	35.4	59.1	40.4	50.0	63.0	67.0	75.5
Moyenne		38.59	37.93	76.13	32.71	48.21	51.03	64.66	93.49

Note: Nymphose à 75°F. et 85% R.H.
† *Immersions à 34°F. seulement.*
‡ *Immersions à 75°F. seulement.*

TABLEAU XXI

ANALYSE DE VARIANCE SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau XX)

Sources de variation	*D.L.*	Variance
Blocs	1	124.60
Jours	3	449.99
Traitements	7	3588.01**
Erreur	52	258.39
Total	63	Erreur standard = 16.07

** *Dépasse "erreur" variance, niveau significatif de 1%.*

6. Lorsque les cocons sont immergés dans les solutions d'acide sulfurique durant l'hibernation seulement, le taux de mortalité est de 32.7%, contre 48.2% si l'immersion ne se produit que durant la nymphose. Ceci, encore, confirme ce que nous avons déjà constaté dans le cas des immersions dans l'eau distillée, à savoir que les larves succombent en plus grand nombre si les cocons sont en contact avec l'eau durant la nymphose que durant la diapause.

Émergence

Dans plusieurs cas, les immersions durant l'hibernation dans l'eau et les solutions d'acide sulfurique, de même que les immersions durant l'hibernation et la nymphose dans l'acide sulfurique réduisent la diapause d'un mois. En effet, alors qu'il n'y eut aucune émergence chez les lots placés dans l'incubateur de 75°F. et 85% R.H. après huit semaines d'hibernation sans contact avec l'eau, pour les trois traitements mentionnés plus haut, nous avons respectivement 5, 6, et 8% d'émergence. Dans le cas d'immersions de deux heures à 34° et 75°F. dans des solutions d'acide sulfurique, nous avons donc eu presque autant d'émergence après huit semaines d'hibernation qu'après 12 semaines lorsque les cocons ne sont pas venus en contact avec des solutions aqueuses.

A trois exceptions près, nous n'avons noté aucune reprise de développement après huit semaines à 34°F. pour tous les autres types d'immersion. C'est pourquoi dans les remarques qui suivent, je considère l'effet de différents traitements sur la rupture de la diapause seulement après 12 et 16 semaines d'hibernation.

Vu que dans presque tous les cas où il y a eu reprise de développement, il en est résulté l'éclosion de l'adulte du cocon, le taux d'émergence a été utilisé comme indice d'efficacité de tel ou tel type d'immersion sur la réactivation des larves. Dans de rares cas, bien qu'il y a eu rupture de la diapause et de la nymphose, les conditions de milieu étant défavorables aux pupes et adultes nouvellement formés, il en est résulté une mortalité anormale durant ces deux stages. Ceci s'est présenté notamment après 8 et 12 semaines chez les lots subissant des immersions à 34° et 75°F., dans des solutions de glycocolle à pH 5. Dans le cas des lots ayant passé 12 semaines en hibernation, les sujets morts à l'état de pupe ou d'adulte dans le cocon représentaient 23% des individus vivants et éclos à la fin des expériences.

Pour éviter toute longueur inutile, résumons ensemble les résultats obtenus après des immersions dans l'eau et dans des solutions acides, tels que présentés et analysés aux Tableaux XXII à XXVII.

1. Dans tous les cas, le nombre de larves qui se nymphosent est plus grand après 16 que 12 semaines d'hibernation.

2. Le taux d'émergence est également plus considérable avec des immersions uniques de deux heures consécutives chaque semaine qu'avec trois immersions hebdomadaires d'une heure chacune.

TABLEAU XXII

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS L'EAU SUR LE POURCENTAGE D'ÉMERGENCE

Blocs	Jours à 34°F.	Imm. à 34°F.		Imm. à 75°F.		Imm. à 34°F. et 75°F.	
		2 hres	3 hres	2 hres	3 hres	2 hres	3 hres
I	84	21.4	4.1	6.5	3.6	0	0
	112	10.9	9.8	17.4	5.9	14.3	5.8
II	84	17.2	13.2	0	3.8	0	0
	112	26.8	10.6	7.1	9.8	11.4	4.3
Moyenne		19.1	9.4	7.8	5.8	6.4	2.5

Note: Nymphose à 75°F. et 85% R.H.

TABLEAU XXIII

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau XXII)

Sources de variation	*D.L.*	Variance
Blocs	1	0.84
Traitements	11	84.91*
Jours	1	172.27*
Immersions	2	209.13*
Heures	1	160.85*
Jrs × Imm.	2	37.50
Jrs × Hres	1	18.74
Imm. × Hres	2	31.80
Jrs × Imm. × Hres	2	12.72
Erreur	11	23.93
Total	23	Erreur standard = 4.89

Différences significatives: Jours et heures = 4.41
Immersions = 5.41

* *Dépasse "erreur" variance, niveau significatif de 5%.*

TABLEAU XXIV

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE D'ÉMERGENCE

Blocs	Jours à 34°F.	H_2SO_4		NH_2CH_2COOH	
		pH 5	pH 3	pH 5	pH 3
I	84	9.3	30.6	6.8	0
	112	29.5	34.8	25.6	0
II	84	2.0	24.1	7.1	0
	112	26.3	26.7	16.2	6.1
Moyenne		16.5	29.0	13.9	1.5

TABLEAU XXV

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau XXIV)

Sources de variation	*D.L.*	Variance
Blocs	1	49.35
Traitements	7	320.01**
Jours	1	454.75**
Acides	1	922.64**
pH	1	0.02
Jrs × Ac.	1	18.71
Ac. × pH	1	608.86**
Jrs × pH	1	221.28**
Jrs × Ac. × pH	1	13.79
Erreur	7	14.45
Total	15	Erreur standard = 3.801

** *Dépasse "erreur" variance, niveau significatif de 1%.*

TABLEAU XXVI

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE D'ÉMERGENCE

Blocs	Jours à 34°F.	H_2SO_4					NH_2CH_2COOH		
		pH 5	pH 3				pH 5	pH 3	
		2 hres	2 hres	3 hres	2 hres†	2 hres‡	2 hres	2 hres	3 hres
I	84	9.3	30.6	0	21.3	0	6.8	0	0
	112	29.5	34.8	21.6	23.6	17.9	25.6	0	3.7
II	84	2.0	24.1	0	13.7	2.5	7.1	0	0
	112	26.3	26.7	20.5	32.2	8.9	16.2	6.1	0
Moyenne		16.8	29.0	10.5	22.7	7.3	13.9	1.5	0.9

† *Immersion à 34°F. seulement.*
‡ *Immersion à 75°F. seulement.*

TABLEAU XXVII

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau XXVI)

Sources de variation	*D.L.*	Variance
Blocs	1	46.08
Jours	1	970.21**
Traitements	7	389.95**
Erreur	22	30.09
Total	31	Erreur standard = 5.49

** *Dépasse "erreur" variance, niveau significatif de 1%.*

3. Lorsque les immersions se font dans l'eau pure, il n'y a pas de différence marquée dans la reprise du développement, que ces immersions se produisent durant la nymphose seulement ou bien durant l'hibernation et la nymphose; 6.8 et 4.5% d'émergence. Par contre, si les immersions ont lieu à 34°F. seulement, la diapause est plus facilement rompue et l'émergence est alors de 14.3%.

4. Lorsque les immersions se font dans une solution d'acide sulfurique, les résultats sont tout autres. D'abord si elles ont lieu à 75°F. seulement, le taux d'émergence (7.3%) est nettement inférieur à celui des immersions à 34°F., de même qu'à celles de 34° et 75°F. (Tableau XXVI). Ensuite, la réactivation maximum n'est pas obtenue ici après les immersions à 34°F., avec 22.7% d'émergence, mais bien après le traitement comportant des immersions à 34° et 75°F., avec 29% d'émergence. Cependant, cette différence entre les deux traitements n'est pas assez considérable pour démontrer la supériorité d'un traitement sur l'autre.

5. Après deux heures par semaine de séjour dans l'eau à 34°F., suivi d'un stage de 60 jours en incubateur, 19.1% des larves se nymphosent, contre 10.6% seulement s'il n'y a pas eu immersion à 34°F. (Tableau V*). Les erreurs standards des deux groupes d'expériences (Tableaux XXIII et VI*) sont 4.89 et 4.10 avec un minimum de sept degrés libres. Pour être significative la différence entre nos deux moyennes devrait être d'au moins:

$$2.36\sqrt{\left(\frac{4.89}{\sqrt{4}}\right)^2+\left(\frac{4.10}{\sqrt{4}}\right)^2} = 7.5$$

Il n'y a donc pas de doute qu'après des immersions de deux heures par semaine durant l'hibernation, la diapause est plus facilement rompue que si le cocon ne vient pas en contact avec l'eau.

Si l'on compare maintenant les résultats obtenus après deux heures d'immersions dans l'eau pure à 34°F., avec ceux de deux heures d'immersions à 34° et 75°F., dans des solutions d'acide sulfurique à pH 3 on observe une différence de (29 – 19.1) 9.9% dans le nombre d'individus qui reprennent leur développement lorsque placés par la suite en incubateur. Nous avons une différence significative lorsqu'elle est supérieure à:

$$2.20\sqrt{\left(\frac{5.89}{\sqrt{4}}\right)^2+\left(\frac{4.89}{\sqrt{4}}\right)^2} = 8.4$$

Les immersions dans des solutions d'acide sulfurique à pH 3 durant l'hibernation et la nymphose provoquent donc une réactivation plus grande que les immersions dans l'eau pure.

6. La variance pour les acides, Tableau XXV, est supérieure au niveau significatif de 1%, démontrant ainsi la supériorité de l'acide sulfurique sur le glycocolle. Cependant, lorsque le pH n'est que de 5, la concentration n'est pas suffisante pour mettre en évidence l'action contraire des deux acides. C'est pourquoi au Tableau XXVI, la différence de développement pour les

* *Dans la Partie I.*

deux solutions à pH 5 n'est pas significative. Mais à un pH de 3, après deux heures d'immersion par semaine à 34° et 75°F., le contraste est frappant, soit 29% d'émergence pour l'acide sulfurique et 1.5% seulement pour le glycocolle.

7. Les chiffres du Tableau XXVI montrent que le pH agit fortement sur la rupture de la diapause; le taux d'émergence étant de 16.8% après immersion dans une solution d'acide sulfurique à pH 5, et 29% après immersion à pH 3. Il est vrai que d'après notre analyse du Tableau XXV, l'effet du pH serait nul, mais dans ce cas notre analyse, du moins en ce qui concerne le pH, est faussée par les résultats du glycocolle à pH 3 où le développement fut à peu près nul. C'est pour des cas de ce genre (Tableau XXIV), que Cochran (29) écrivait que lorsque les différences entre différents traitements sont de plus de 100% ou lorsqu'il y a insuccès partiel de certains traitements, il valait mieux dans l'analyse de variance d'omettre ces traitements. Comme règle générale dans les analyses de routine, Cochran suggère d'omettre les traitements dont les résultats sont continuellement plus du double ou de moins de la moitié du groupe principal de traitements. Il aurait donc été préférable d'éliminer du Tableau XXIV la colonne du glycocolle à pH 3. J'ai conservé cette colonne pour deux raisons: d'une part, afin de ne pas enlever la symétrie du tableau, ce qui aurait rendu impossible l'analyse de variance pour d'autres sources de variation qui ne sont pas faussées par le peu de développement dans le glycocolle à pH 3; d'autre part, pour illustrer comment on peut arriver à de fausses interprétations en faisant des analyses de variance de façon mécanique. Au Tableau XXVIII, les résultats avec les solutions de glycocolle ont été éliminés et le pH nous donne alors une variance très significative, mettant ainsi en évidence l'influence du pH sur la réactivation des larves.

TABLEAU XXVIII

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Basée sur Colonnes 3 et 4 du Tableau XXIV)

Sources de variation	*D.L.*	Variance
Blocs	1	78.82
Traitements	3	252.68*
Jours	1	329.42*
pH	1	301.42*
Jours × pH	1	127.21
Erreur	3	18.95
Total	7	Erreur standard = 4.353

** Dépasse "erreur" variance, niveau significatif de 5%.*

8. D'après l'interaction jours sur pH, Tableau XXV, le développement après 84 jours d'hibernation serait plus considérable à pH 3 qu'à pH 5, tandis qu'après 112 jours un pH de 3 diminuerait l'émergence. Ici, encore, l'analyse est faussée par les résultats du glycocolle à pH 3 qui n'auraient pas dû être considérés. En effet, l'analyse du Tableau XXVIII nous prouve que l'interaction jours sur pH n'influence en aucune façon le pourcentage d'émergence.

Action d'immersions dans l'acide sulfurique concentré

Les expériences décrites dans ce chapitre avaient pour but de déterminer l'influence que peut avoir sur la rupture de la diapause: (*a*) une dessication rapide au début de l'hibernation, et (*b*) l'action de chocs chimiques.

A cet effet, avant ou après les périodes à 34°F., périodes variant comme d'habitude de 42 à 116 jours, les traitements suivants furent expérimentés:

e. Immersion de trois minutes dans une solution à 95% d'acide sulfurique au début de l'hibernation suivie, durant la nymphose, du traitement *b* comportant trois immersions dans l'eau d'une heure chacune par semaine pour *Diprion*, et du traitement *d*, soit six heures d'immersion pour *Pristiphora*.

f. Immersion de trois minutes dans l'acide sulfurique concentré à la fin de l'hibernation sans immersion dans l'eau à 75°F. par la suite.

g. Immersion de trois minutes dans l'acide sulfurique à la fin de l'hibernation, suivie des traitements *b* et *d* durant la nymphose.

h. Même traitement que *e* mais avec une durée d'hibernation de 112 jours.

i. Immersion d'une minute dans l'acide sulfurique à la fin de l'hibernation. Aucun contact avec l'eau dans l'incubateur.

j. Immersion de deux minutes dans l'acide sulfurique à la fin de l'hibernation. Aucun contact avec l'eau à 75°F.

Je n'avais d'abord prévu dans mon programme que les immersions *e*, *f*, et *g*, mais vu le fort pourcentage de mortalité qui en résulta, les traitements *f* et *g* furent changés pour *i* et *j* pour les lots subissant 16 semaines à 34°F.

Résistance à l'acide sulfurique concentré

Avant d'adopter les immersions de trois minutes, j'ai auparavant déterminé la résistance de *Diprion* dans l'acide sulfurique concentré. Dix-huit lots de 25 cocons chacun furent d'abord placés dans nos tubes de verre de 25 × 90 mm. Ces tubes furent ensuite plongés individuellement, pour des périodes variant de 1 à 12 minutes, dans un bocal rempli d'acide sulfurique à 95% de façon à ce que les cocons soient complètement submergés par l'acide. Une fois l'immersion terminée, les cocons furent lavés à l'eau, puis immergés pour une heure dans un autre bassin rempli d'eau et finalement transférés pour 24 heures, soit dans l'incubateur à 75°F. et 85% R.H., soit dans le compartiment à 34°F. de notre réfrigérateur. Après ce délai, ils furent ouverts et l'état des larves noté. Les résultats obtenus dans ces expériences sont présentés au Tableau XXIX.

TABLEAU XXIX

RÉSISTANCE DE *Diprion polytomum* DANS L'ACIDE SULFURIQUE CONCENTRÉ

Minutes dans H_2SO_4 conc.	% de mortalité après un stage de 24 heures à:		Minutes dans H_2SO_4 conc.	% de mortalité après un stage de 24 heures à:	
	34°F.	75°F. et 85% R.H.		34°F.	75°F. et 85% R.H.
1	16	40†	6	20	44
2	20	28	8	40	64
3	16	16	10	52	84
4	16	28	12	32	84
5	20	20			

† *Dans ce lot, 24% des larves étaient mortes avant l'expérience.*

Après 24 heures à 34°F. le nombre de larves mortes n'est pas anormal, même si l'immersion a duré jusqu'à 12 minutes. Par contre, après une immersion de 10 minutes dans l'acide on enregistre une mortalité de plus de 80% dans les cocons, 24 heures après qu'ils ont été placés à 75°F. Il n'y a pas de doute qu'en dépit du lavage dans l'eau, il reste toujours une certaine quantité d'acide d'imprégné dans le cocon. Il faut croire qu'à une basse température, l'action de cet acide est presque nulle, mais à une température élevée et dans un milieu très humide, il continue à agir sur la larve et entraîne une plus forte mortalité.

Au début de mes expériences, j'avais décidé d'adopter pour la durée de mes immersions, la moitié de la période requise pour qu'il y ait mortalité notable par l'acide. Des essais préliminaires m'avaient montré que dans le cas des cocons placés en incubateur après leur immersion dans l'acide sulfurique, le taux de mortalité atteignait 44% après six minutes d'immersion, et c'est à ce moment qu'on commençait à constater l'effet mortel de l'acide; c'est pourquoi j'ai choisi des immersions de trois minutes.

Pour *Pristiphora erichsoni*, je ne fis que deux expériences; une immersion de deux minutes et l'autre de trois minutes dans l'acide sulfurique concentré, suivie du lavage dans l'eau et d'un stage de 24 heures dans l'incubateur. Le lendemain sur un total de 40 cocons, il y avait dans un lot 28% des larves de mortes et dans l'autre 32%.

MORTALITÉ DURANT L'HIBERNATION ET LA NYMPHOSE

Les immersions dans l'acide sulfurique concentré au début de l'hibernation n'affectent pas la vitalité de la larve de *P. erichsoni* (Tableau XXX). Ainsi, la moyenne de mortalité à la fin de l'hibernation pour huit lots ayant passé trois minutes dans l'acide sulfurique concentré au début de leur stage à 34°F. fut de 28.2%. Pour 16 lots n'ayant eu aucun contact avec l'acide sulfurique, la moyenne fut de 16.6%, soit une différence de 11.6%. Cette différence, cependant, n'est pas suffisante pour indiquer une action défavorable de l'acide.

TABLEAU XXX

Pristiphora erichsoni, MORTALITÉ DURANT L'HIBERNATION†

Traitements	Moyenne	*E.S.*	$E.S._M$	$E.S._D$	Valeurs de *T*	
					Obtenue	$P = 5\%$
Trois minutes dans H_2SO_4	28.25	14.2	5.02			
Témoin	16.63	17.17	4.29			
Différence	11.62			6.62	1.76	2.36

† *Après trois minutes d'immersion dans l'acide sulfurique concentré.*

car l'erreur standard de la différence n'est que de 6.6 nous donnant une valeur de "t" pour laquelle la probabilité est supérieure à 5%.

Les résultats obtenus pour *Pristiphora* s'appliquent aussi à *Diprion*, les immersions de trois minutes dans l'acide sulfurique n'augmentant pas la mortalité au cours de l'hibernation.

Apparemment, à des températures près du point de congélation, l'acide ne s'infiltre que difficilement dans le cocon et la larve est très peu affectée.

Tous les individus, indistinctement d'espèce, ayant subi trois minutes d'immersion dans l'acide sulfurique au début ou à la fin de l'hibernation, et des immersions dans l'eau durant la nymphose, sont morts après 60 jours en incubateur et même, dans certains cas, après 30 jours seulement d'incubation. Il n'y a aucun doute que pour un bon nombre d'individus les immersions dans l'acide furent mortelles. Ainsi, là où il y avait des faiblesses dans le cocon, dues soit à la manipulation (pressions trop fortes) ou à la texture du cocon lui-même, l'acide sulfurique s'infiltre à l'intérieur et provoque des fissures. Alors les larves étaient totalement décomposées ou encore complètement sorties du cocon. Ailleurs, il y avait évidence que l'acide sulfurique concentré était venu directement en contact avec les larves par les plaques noires qu'elles avaient sur le corps. Cependant des larves de *Diprion*, ayant ainsi tout le dos de l'abdomen (excepté les trois derniers segments) complètement noirci par l'acide sulfurique, étaient encore très vivantes lorsque examinées. En dépit du nombre de larves tuées par l'action directe de l'acide sulfurique, il n'en reste pas moins que les causes de la mort de la grande majorité des individus furent la manipulation et les immersions de trois heures dans l'eau pour *Diprion*, et celles de six heures pour *Pristiphora*. En effet, surtout en ce qui concerne *Pristiphora*, nous avons vu au chapitre précédent qu'après ces immersions nous avions remarqué 100% de mortalité.

Lorsque les immersions de trois minutes dans l'acide sulfurique concentré ne sont pas suivies d'immersions dans l'eau en incubateur, nous avons encore pour *Pristiphora* presque 100% de mortalité après 60 jours à 75°F. et 85%

R.H. Par contre, chez *Diprion* il y eut toujours de 10 à 35% des larves de vivantes à la fin des expériences. L'acide pénètre plus facilement le cocon à texture lâche de *Pristiphora* et le lavage à l'eau après l'immersion est moins efficace. Par la suite lorsque ces cocons sont placés en incubateur, les gaz d'acide sulfurique qui se dégagent à l'intérieur du cocon, tuent au moins 65% des larves après 30 jours, et la quasi-totalité après 60 jours.

Vu le fort pourcentage de mortalité survenu après les immersions de trois minutes dans l'acide sulfurique, je décidai, pour les individus de *Diprion* ayant passé 16 semaines à 34°F., d'éliminer les traitements *f* et *g* pour appliquer les traitements *i* et *j*, soit une et deux minutes d'immersions dans l'acide sulfurique. Les résultats sont présentés au Tableau XXXI, ainsi que ceux obtenus après huit semaines d'hibernation et trois minutes d'immersions dans l'acide sulfurique.

TABLEAU XXXI

Diprion polytomum, POURCENTAGE DE MORTALITÉ ET D'ÉMERGENCE APRÈS IMMERSION DANS L'ACIDE SULFURIQUE CONCENTRÉ

Traitements†	Bloc I		Bloc II		Moyenne	
	Mort.	Émer.	Mort.	Émer.	Mort.	Émer.
e	100		100		100	
f	82	12.5	69	14.3	75.5	12.2
g	98		100		99.0	
h	79		81.3	6.2	80.6	3.0
i	61	62.5	65	48.6	62.5	56.0
j	77	36.4	75	48	76.0	41.7

† *Hibernation de 12 semaines pour e, f, et g; 16 semaines pour h, i, et j.*

Après les trois minutes d'immersion dans l'acide sulfurique au début ou à la fin de l'hibernation, lorsqu'il y eut immersion dans l'eau durant la nymphose, nous avons eu 100% de mortalité pour *e* et *g* et pour le traitement *h* (meilleure manipulation) 80.6%. Lorsqu'il n'y eut pas contact avec l'eau durant la nymphose, traitements *f*, *i*, et *j*, la mortalité fut de: 75.5% après trois minutes d'immersion, 76% après deux minutes, et 62.5% après une minute. Dans ces trois derniers cas, ce n'est pas tant l'acide sulfurique que la manipulation qui fut cause de ce haut pourcentage de mortalité. Car une fois sec, le cocon qui a été plongé dans l'acide sulfurique concentré devient très fragile. A la moindre pression ces cocons s'effritent presque aussi facilement que du papier brûlé. Comme question de fait, à la fin des expériences, il y en avait plusieurs réduits en miettes. Et s'ils n'avaient pas eu à être examinés tous les jours pour l'émergence des adultes, la mortalité aurait été beaucoup moindre.

ÉMERGENCE

Les seuls cocons de la mouche à scie du mélèze contenant des individus vivants à la fin des expériences appartenaient aux lots du traitement *f*, mais aucun adulte n'est éclos de ces cocons.

Des lots de *Diprion* qui, après leur immersion de trois minutes dans l'acide sulfurique, vinrent en contact avec l'eau durant la nymphose, il n'est sorti qu'un seul adulte sur un total de 40 individus vivants.

Lorsque après les bains dans l'acide sulfurique concentré, il n'y eut en incubateur aucune immersion dans l'eau, les résultats furent comme suit (Tableau XXXI): trois minutes d'immersion nous donnent une moyenne de 12.2% d'émergence, ceci toutefois, n'est basé que sur 48 individus vivants et ne représente donc que six adultes. Après deux minutes dans l'acide sulfurique, l'émergence fut de 41.7% aussi sur un total de 48 individus, alors qu'après une minute d'immersion avec 75 individus vivants à la fin de l'expérience, l'émergence monta à 56%.

Bien que le petit nombre d'expériences rendit impossible l'analyse des résultats à l'aide des méthodes statistiques, il est donc fort probable que les immersions dans l'acide sulfurique concentré provoquent une reprise des activités plus considérable que tout autre facteur mis à l'épreuve. Le taux de 56% d'émergence que nous avons eu avec le traitement *i* est à peu près le double du développement obtenu au moyen d'immersions dans des solutions d'acide sulfurique à pH 3 qui, jusqu'ici, s'étaient avérées les plus efficaces. Il est vrai que lorsque les cocons furent en contact avec l'acide sulfurique concentré, la mortalité fut plus élevée que pour tout autre traitement, mais, encore une fois, ceci peut être attribué en partie à la manipulation. De plus, les résultats du Tableau XXXI nous portent à croire qu'un contact de quelques secondes seulement avec l'acide sulfurique activerait une reprise de développement plus considérable et moins de mortalité, car, à mesure que la durée des immersions diminue (de trois à une minute), le pourcentage de mortalité diminue parallèlement alors que l'émergence augmente.

Enfin, on peut conclure que de courtes immersions dans l'acide sulfurique concentré facilitent grandement la rupture de la diapause. Mais leur influence est plutôt le résultat de chocs provoqués à la fin de l'hibernation, qu'à la dessication qu'elles occasionnent au début, car dans de tels cas, il n'y a point ou très peu d'émergence.

Influence du milieu sur la rapidité de nymphose

Nous venons de voir que dans certains milieux la larve offre peu de résistance, et que par le fait même le taux de mortalité est plus élevé. Dans d'autres milieux, au contraire, les larves se nymphosent en grand nombre, mais on observe des différences appréciables dans chaque milieu quant à la vitesse de la rupture de la diapause.

La date d'émergence de chaque adulte ayant été notée, j'ai pu, après avoir groupé ensemble les lots ayant reçu des traitements semblables, établir la distribution de l'émergence pour chacun de ces traitements (Tableaux XXXII et XXXIII). Au moyen des méthodes statistiques, j'ai pu ensuite analyser ces distributions, les comparer et déterminer l'influence de différents milieux sur la rapidité de la rupture de la diapause chez *Diprion polytomum*.

TABLEAU XXXII

ÉMERGENCE DE *Diprion polytomum* APRÈS DIFFÉRENTS TRAITEMENTS

(Saison 1938-1939)

	Température d'hibernation		Température de nymphose		
	(1) 32°F.	(2) 32°F.	(3) 65°F.	(4) 75°F.	(5) 80°F.
Nombre d'individus éclos	266	93	34	146	91
Date moyenne d'éclosion	34.87	36.52	53.56	35.2	29.6
E.S.	12.3	11.0	11.25	9.3	10.1
$E.S._M$	0.75	1.14	1.93	0.77	1.06

TABLEAU XXXIII

ÉMERGENCE DE *Diprion polytomum* APRÈS DIFFÉRENTS TRAITEMENTS

(Saison 1940-1941)

	Hibernation en jours		Température d'hibernation		Humidité relative de nymphose		Variations brusques de température	
	(1) 84	(2) 112	(3) 45°F.	(4) 34°F.	(5) 75%	(6) 56%	(7) *E*	(8) *F*
Nombre d'individus éclos	83	92	13	31	23	19	38	24
Date moyenne d'éclosion	22.55	21.42	32.53	18.87	19.69	20.37	16.98	16.62
E.S.	5.07	6.42	9.45	2.06	8.22	8.67	2.42	4.32
$E.S._M$	0.551	0.64	2.63	0.37	1.72	1.99	0.39	0.88

	Immersions dans l'eau			
	(9) à 34°F.	(10) à 75°F.	(11) 2 hres	(12) 3 hres
Nombre d'individus éclos	50	34	78	29
Date moyenne d'éclosion	22.86	28.59	25.03	26.88
E.S.	11.172	8.52	7.74	8.142
$E.S._M$	1.58	1.46	0.87	1.39

	Immersions dans solutions acides			(16) Immersions dans H_2SO_4 conc.
	H_2SO_4		(15) Glycocolle à pH 5	
	(13) pH 5	(14) pH 3		
Nombre d'individus éclos	38	33	20	49
Date moyenne d'éclosion	22.19	22.91	25.05	28.41
E.S.	8.07	3.24	9.36	4.26
$E.S._M$	1.29	0.56	2.09	0.60

Le Tableau XXXII est basé sur les émergences de 1938-1939, alors que le Tableau XXXIII contient les résultats de mes expériences de 1940. Il aurait été intéressant de comparer les résultats de certains traitements rapportés dans ces deux tableaux; par exemple la rapidité d'émergence dans un milieu sec (Tableau XXXII) et dans un milieu humide (Tableau XXXIII). La chose fut impossible parce que la différence alors aurait pu être due non pas aux facteurs mis en cause, mais à des variations occasionnées par suite du fait que le matériel employé avait été récolté en deux années différentes.

TABLEAU XXXIV

ANALYSE STATISTIQUE SUR L'INFLUENCE DU TRAITEMENT SUR LA RAPIDITÉ DE LA NYMPHOSE

(Voir Tableau XXXII)

Traitements comparés	Différence	*E.S.d*
4 et 5	1.65	1.36
6 et 7	18.36**	2.08
7 et 8	5.60**	1.31

** *Dépasse le niveau significatif de 1%.*

TABLEAU XXXV

ANALYSE STATISTIQUE SUR L'INFLUENCE DU TRAITEMENT SUR LA RAPIDITÉ DE LA NYMPHOSE

(Voir Tableau XXXIII)

Traitements comparés	Différence	*E.S.d*	Traitements comparés	Différence	*E.S.d*
1 et 2	1.13	0.84	9 et 10	5.73*	2.14
3 et 4	16.66**	2.66	11 et 12	1.85	1.64
5 et 6	0.68	2.63	13 et 14	1.72	1.41
5 et 7	2.71	1.76	13 et 15	2.86	2.46
5 et 9	3.17	2.32	13 et 16	6.22**	1.43
7 et 8	0.36	0.93	15 et 16	3.36	2.18

* *Dépasse le niveau significatif de 5%.*
** *Dépasse le niveau significatif de 1%.*

De l'interprétation de ces distributions de l'émergence analysées aux Tableaux XXXIV et XXXV on peut déduire que:

1. La rapidité de nymphose est indépendante de la température d'hibernation, si celle-ci se produit à des températures voisines du point de congélation, ou inférieure à celui-ci, soit de 32° à 15°F. Si l'hibernation a lieu à des températures relativement élevées, par exemple 45°F., la reprise des activités est alors beaucoup plus lente; la moyenne d'émergence est alors de 32.5 jours, contre 18.9 jours à 34°F.

2. La rupture de la diapause n'est pas plus rapide après 12 semaines d'hibernation qu'après 16 semaines.

3. La vitesse de développement est en rapport direct avec la température de nymphose, augmentant avec celle-ci, jusqu'à ce que la limite vitale supérieure soit atteinte. Comme on pouvait s'y attendre la métamorphose est beaucoup plus lente à 65°F. Mais même entre les températures de 75° et 80°F., on constate un retard significatif de 5.6 jours à 75°F.

4. Les changements brusques des températures d'hibernation à celles de nymphose n'ont aucune influence sur la vélocité du développement.

5. L'humidité relative de l'incubateur lorsqu'elle varie entre 85 et 56% ne semble avoir aucune influence sur la vitesse de la reprise du métabolisme.

6. En général, les immersions dans l'eau favorisent un plus grand développement, mais dans certains cas, elles retardent la réactivation. Après les immersions durant l'hibernation seulement, les larves reprennent leur évolution avec un retard de faible amplitude qui, d'ailleurs, est sans importance comme l'indiquent les traitements 5 et 9, inclus dans le Tableau XXXV. Au contraire, lorsque les immersions ont lieu durant la nymphose, le retard est alors plus considérable et significatif, tel que le montrent les traitements 9 et 10, du Tableau XXXV.

7. Après des immersions de trois heures par semaine dans l'eau, l'émergence présente un retard de deux jours sur les immersions de deux heures par semaine, mais cette différence est négligeable.

8. Le pH n'a aucune influence sur la rapidité de nymphose. Après des immersions dans des solutions d'acide sulfurique à pH 3, la métamorphose survient presque en même temps; les dates moyennes d'émergence étant de 22.2 et de 22.9 jours. Ces chiffres nous montrent aussi que la réactivation est aussi rapide après les immersions dans l'acide sulfurique que dans l'eau, dans ce dernier cas, l'émergence moyenne étant de 22.9 jours.

9. On a vu que les solutions de glycocolle à pH 5 n'augmentent pas la mortalité de façon notable. Elles n'influencent pas non plus la rapidité de la réactivation. A un pH de 5, la solution n'est pas assez concentrée pour retarder la rupture de la diapause et la plupart des adultes apparaissent après 25 jours soit trois jours plus tard qu'après les immersions dans l'acide sulfurique à pH 5, ce qui n'est pas significatif.

10. Après les immersions d'une et deux minutes dans l'acide sulfurique concentré à la fin de l'hibernation, les larves reprennent leur évolution avec un retard de six jours sur les immersions dans l'acide sulfurique à pH 5, et d'environ neuf jours sur les individus n'ayant subi aucune immersion. La reprise du développement est donc beaucoup plus lente après les chocs provoqués par l'acide sulfurique concentré.

Discussion et conclusions

Les Tenthrèdes faisant l'objet de la présente étude exhibent des diapauses de durées très variables. Chez *Diprion polytomum*, espèce sur laquelle a porté la plus grande partie de mes observations, la larve peut demeurer à

l'état de vie latente dans son cocon pendant des périodes variant de 10 jours à 7 ans. Des irrégularités aussi considérables dans la durée de la diapause ne peuvent s'expliquer simplement par l'action de facteurs ou agents n'agissant sur les individus qu'au cours de leur séjour dans leur cocon. Si tel était le cas, il ne serait pas après tout très difficile de mettre ces facteurs en évidence, et de briser ces longs sommeils, alors qu'au contraire dans bien des cas la chose est impossible.

Apparemment, pour résoudre la question de la diapause chez les Tenthrèdes, et plus particulièrement chez la mouche à scie européenne de l'épinette, il faudrait suivre les sujets en expérience avant même leur naissance, commencer dès l'éclosion des parents, connaître le nombre de jours et les conditions dans lesquelles la mère a vécu avant la ponte. Il faudrait aussi déterminer quelles sont les conditions optima pour l'incubation et le développement larvaire, et faire l'élevage des sujets dans ces conditons. Alors seulement nous pourrions espérer d'éliminer complètement la diapause. Puis, pour en déterminer les causes, il nous faudrait varier ces conditions idéales de milieu en commençant par un facteur à la fois, puis ensuite avec plusieurs en même temps. En connaissant les causes de la diapause, il nous serait alors plus facile de trouver les moyens pour y mettre fin. En un mot, il nous faudrait répéter avec *D. polytomum* les expériences exécutées par Cousin (30) avec *Lucilta sericata*. Ceci représenterait un travail de longue durée, mais dont les résultats seraient des plus intéressants. Nous ne perdons pas l'espoir de l'entreprendre sous peu. Le point faible du travail de Cousin provient du fait qu'elle a utilisé dans ses expériences, des espèces polyvoltines dont la diapause normalement n'est pas très prononcée, ni de longue durée et beaucoup plus facile à rompre que pour des espèces univoltines à longues diapauses, comme c'est le cas pour *Diprion*. C'est pourquoi, certains auteurs ont mis en doute certaines des conclusions de Cousin, car ils ne veulent pas considérer comme diapause un simple ralentissement dans le métabolisme, ralentissement cessant facilement lors du retour aux conditions normales. Mais si les expériences de Cousin étaient répétées avec un insecte comme *Diprion*, et que les résultats de cet auteur puissent être confirmés, comme je crois que ce serait le cas, il me semble que ceci serait suffisant pour prouver le bien-fondé de sa théorie.

Tout en faisant certaines restrictions pour les raisons ci-haut mentionnées, il n'est pas douteux que quel que soit le milieu dans lequel s'est effectué le développement embryonnaire ou larvaire, certains facteurs ont une action générale bien définie sur la rupture de la diapause. Ce fut le principal objet du présent travail de déterminer, au moyen d'études expérimentales, quelles sont les conditions qui, à l'automne, durant l'hiver et au printemps, peuvent augmenter ou diminuer la reprise du métabolisme chez les Tenthrèdes.

Les résultats essentiels de mes expériences et leur application dans leur milieu naturel me permettent de préciser les points suivants:

1. La différence observée dans le taux de mortalité après des hibernations à des températures variant de 45° à 15°F. est tout à fait insignifiante. *Diprion*,

de même que *Pristiphora*, résiste aussi bien à 15° qu'à 45°F. Cependant un stage assez long à 15°F. empêche ultérieurement la reprise des activités lorsque les conditions redeviennent favorables à la nymphose et, de plus, diminue la résistance des individus quand ceux-ci sont exposés à 75°F. et 85% R.H. Il semble donc que si, tard à l'automne, avant que le sol soit recouvert d'une couche de neige assez épaisse, la température du sol descend aux environs de 15°F. et s'y maintient assez longtemps, il peut en résulter une réduction dans le pourcentage d'émergence l'année suivante, et une mortalité anormale au début de l'été suivant. D'après Cousin (30), il ne faut pas toujours attribuer cette persistance de la diapause à des troubles causés directement par le froid, mais à la déshydratation qui résulte d'une chûte thermique progressive. Dans plusieurs cas, la rupture de ces diapauses est alors possible par une exposition à une température chaude et *humide*. Quant au taux de développement après l'hibernation à 45° et 34°F., il est aussi considérable dans un cas comme dans l'autre, mais après une hibernation à 45°F., la réactivation est plus lente qu'après une hibernation à 34°F.

2. D'après Balch (7) quelques larves pourraient survivre à des hibernations à −1°F., alors que toutes mourraient à −20°F. Malheureusement, cet auteur n'indique pas la durée d'exposition nécessaire pour provoquer la mortalité à ces deux températures. Dans mes expériences aucune des espèces étudiées n'a pu vivre très longtemps à 0°F. *Diprion polytomum* n'a pas résisté plus de 10 semaines à cette température. Il en fut de même pour *Pristiphora erichsoni*, *Hylotoma pectoralis*, et *Neodiprion pinetum*. Il faut admettre, cependant, que des températures aussi basses se rencontrent rarement lorsque les larves se trouvent dans leur milieu naturel.

3. Lorsque la nymphose se fait dans un milieu sec, soit environ 56% R.H., la mortalité est plus élevée si l'hibernation s'effectue à des températures voisines ou inférieures au point de congélation, que si elle se produit à des températures bien supérieures à ce point.

4. Chez toutes les Tenthrèdes étudiées, après une durée minimum de 8 à 10 semaines à 45° et 34°F., il y a reprise du développement à des températures aussi basses que 60°F., et dans un milieu très sec. Mais, il va sans dire que le taux d'émergence est alors bien inférieur et moins rapide que celui que l'on obtient à des températures plus élevées. La rapidité de la reprise du métabolisme est en relation directe avec la température de nymphose, augmentant avec celle-ci.

5. Durant l'incubation, l'humidité relative plus que la température influence la résistance des individus. Ainsi une température de 85°F., associée à une atmosphère sèche, est nettement fatale aux Tenthrèdes; la plupart meurent en moins d'un mois. Tout au contraire, si l'humidité relative est élevée, les larves résistent mieux à cette même température qu'à la température optimum de 75°F., associée à une atmosphère sèche. D'un autre côté, lorsqu'il s'agit de la rupture de la diapause la température semble plus importante que l'humidité relative. A 85°F., même en milieu humide, la

larve reste en diapause, alors qu'à 75°F. et 85% R.H., la réactivation est aussi intense et aussi rapide qu'à la même température avec seulement 56% R.H.

6. Dans un milieu très sec, 20 à 30% R.H., le pourcentage d'adultes mourant dans leur cocon peut atteindre jusqu'à 50%, tout simplement parce que celui-ci est trop dur et sec pour que les adultes puissent couper leur trou de sortie. Il faut dire, cependant, que dans la nature de telles conditions hygrométriques doivent se rencontrer rarement.

7. A 90°F. et 20 à 25% R.H., aucune Tenthrède ne peut demeurer vivante dans son cocon plus d'une semaine. Après ce stage, les larves sont devenues brunes et dures, présentant la même apparence que celles dont les cocons durant l'été sont directement exposés aux rayons du soleil ou protégés seulement par une légère couche de mousse ou d'humus. On observe cet état de chose durant l'été dans les jeunes peuplements originant d'un feu ou d'un terrain déjà cultivé où le sol est très dur et où la couche d'humus est à peu près inexistante; les cocons se trouvant alors presque à la surface du sol. Dans d'autres peuplements d'épinette, on rencontre quelquefois des éclaircies constituées par des rochers recouverts d'une mince couche de matière végétale, sous laquelle nombre de larves cherchent refuge pour y tisser leur cocon. Durant l'été, la température sur ces rochers peut dépasser de beaucoup 90°F., et les individus qui ne sont pas éclos de bonne heure à l'été sont généralement tués plus tard par l'action de la chaleur.

8. Si l'on ne considère que les facteurs température et humidité relative, nous aurons la mortalité minimum et le maximum de développement après une hibernation aux environs du point de congélation et une nymphose à 75° ou 80°F., associée à une humidité relative de 85% ou plus.

9. Plusieurs auteurs notamment Roubaud (92), Parker (68), Cousin (30), et Balachowsky (6), ont noté l'influence de chocs thermiques sur la rupture de la diapause. Les expériences que j'ai faites à ce sujet sur *Diprion polytomum* permettent de distinguer deux séries de résultats. D'abord les variations brusques de température au début ou à la fin de l'hibernation ne réduisent pas la vitalité de *Diprion* lors de la période de nymphose. Ensuite les chocs thermiques au début de l'hibernation provoquent des diapauses plus difficiles à rompre. Au contraire, les mêmes chocs thermiques, s'ils ont lieu à la fin de l'hibernation entraînent une réactivation bien supérieure à celle obtenue après des changements graduels de température. Ces chocs, cependant, n'accélèrent pas la reprise du métabolisme. Si on interprète ces résultats, on peut estimer qu'à l'automne des changements subits et à grandes amplitudes dans la température pourraient réduire le pourcentage d'émergence l'année suivante, alors qu'au printemps, les mêmes changements subits amèneraient la nymphose d'un plus grand nombre de larves.

10. Les chocs provoqués par les immersions dans l'acide sulfurique concentré ont un effet analogue aux chocs thermiques, mais encore plus accentué que celui causé par ces derniers. Lorsque les immersions dans l'acide se font au début de l'hibernation, il n'y a point ou très peu d'émergence. Il doit alors se produire durant l'hibernation une forte dessication rendant la rupture

de la diapause très difficile, c'est-à-dire qu'ici l'acide sulfurique jouerait le même rôle que les froids excessifs. Par contre, après des immersions d'une minute à la fin de l'hibernation, l'émergence est presque deux fois plus considérable qu'après des immersions régulières de deux heures par semaine dans des solutions aqueuses. Les immersions dans l'acide sulfurique concentré raniment donc un grand nombre de larves qui autrement resteraient en diapause, mais cette reprise du métabolisme se fait avec un retard de six jours sur les immersions dans les solutions aqueuses à pH 5 et de près de neuf jours sur les individus n'ayant subi aucun contact avec l'eau.

11. A des températures voisines du point de congélation, les larves en diapause peuvent vivre plus de trois semaines complètement submergées dans l'eau, tandis qu'à 75°F., la larve ne peut supporter beaucoup plus qu'une semaine d'immersion. Dans nos forêts la température dans la mousse et l'humus n'atteint à peu près jamais 75°F. Mais il est probable que durant l'été, un sol saturé d'eau pendant plusieurs jours est fatal à un bon nombre de larves dans leur cocon, sans causer toutefois une mortalité de 100%.

12. Apparemment il y aurait plus de mortalité et moins d'émergence, après des immersions fréquentes mais de courtes durées, qu'après des immersions plus longues mais moins rapprochées. Cependant les résultats de ces expériences ne sont pas très concluants parce que dans le premier cas le taux de mortalité plus élevé peut, en partie, être attribuable à la manipulation.

13. Les immersions de deux ou trois heures par semaine durant l'hibernation et la nymphose entraînent la mort d'un plus grand nombre d'individus que si ces bains ne se produisent que durant l'hibernation. Il semble, de plus, que ce soit le contact de l'eau durant la nymphose qui soit particulièrement défavorable, la mortalité étant presque aussi élevée si les immersions n'ont lieu qu'à 75°F., que si elles se sont produites durant le séjour de la larve à 34° et à 75°F. Si l'on considère maintenant le développement, on remarque que lorsque les immersions dans l'eau ne se produisent que durant la nymphose, le taux d'émergence est inférieur à celui obtenu après des bains à 34°F. seulement, ou encore à 34° et 75°F. Dans ces deux derniers cas, le taux de développement ne diffère pas sensiblement. Ce serait donc les immersions durant l'hibernation qui activeraient surtout la reprise du développement. Babcock (3 et 4) lors de ses expériences sur la pyrale du maïs (*Pyrausta nubilalis*) remarqua que cet insecte est très sensible au contact de l'eau durant l'hibernation. S'il y a alors dessication, il en résulte un retard dans la pupaison ou diapause prolongée. Peut-être est-ce la même chose avec *Diprion polytomum*. Chose certaine c'est qu'après des immersions à 34°F., le métabolisme est beaucoup plus intense lors de l'exposition à 75°F. que si les individus n'ont eu aucun contact avec l'eau durant l'hibernation.

14. L'acidité du sol n'affecte en aucune façon le bien-être des larves dans leur cocon. Au contraire, elle active le métabolisme et à mesure que le pH diminue, le développement augmente. Les immersions dans les solutions d'acide sulfurique à pH 3 durant l'hibernation et la nymphose provoquent une réactivation plus grande que les immersions dans l'eau pure. D'après ces

résultats il ne semble pas y avoir de doute que dans les sols très acides le taux d'émergence devrait être plus considérable. Sautet (104) avait lui aussi constaté que certains composés chimiques amènent une réactivation rapide des larves en diapause d'*Anopheles bifurcatus*. Dans ses expériences ce rôle déclanchant était joué par des oxydants: eau de javel et permanganate de potassium, tandis que dans la nature, dit-il, "c'est l'oxygène provenant de la fonction chlorophyllienne des plantes aquatiques qui agit comme oxydant."

15. Les immersions dans le glycocolle sont néfastes aux larves dans leur cocon. Même durant l'hibernation leur influence se fait déjà sentir et lorsque les immersions se poursuivent durant la nymphose le pourcentage de mortalité devient alors considérablement plus élevé qu'après les immersions dans l'eau ou les solutions d'acide sulfurique. De plus le glycocolle inhibe considérablement la réactivation et lorsqu'il y a nymphose, le taux de mortalité à l'état de pupe ou d'adulte non éclos est plus élevé que dans tout autre milieu. Le glycocolle semble donc toujours toxique aux larves de *Diprion* dans leur cocon, et si dans les sols forestiers, il peut y avoir des concentrations de glycocolle ou même d'acides aminés en général aussi grandes que celles employées dans nos solutions aqueuses, on devrait s'attendre à un taux de mortalité plus élevé et à des diapauses plus longues.

16. Les immersions dans l'eau ou des solutions acides réduisent la durée de la diapause d'un mois. Dans le cas d'immersions durant l'hibernation et la nymphose dans des solutions d'acide sulfurique à pH 3 par exemple, nous avons presque autant d'émergence après huit semaines d'hibernation qu'il y en a après 12 semaines lorsque les cocons ne sont pas venus en contact avec des solutions aqueuses. Cependant, il reste toujours, même dans les lots ayant subi des immersions, que le nombre de larves qui se nymphosent est plus grand après 16 qu'après 12 semaines d'hibernation.

Chez des espèces ayant normalement des diapauses de plusieurs mois, voire même de plusieurs années, et cela depuis nombre de générations, on peut difficilement s'attendre qu'il soit possible de briser ces diapauses après un séjour de courte durée au froid. La chose devient d'autant moins probable lorsque les sujets ont vécu dans des milieux variables, non contrôlés et souvent défavorables, comme c'est le cas lorsqu'ils proviennent de leur milieu naturel. Il n'y a pas de doute alors que le facteur temps est important. Et même si les conditions de nymphose sont idéales, il ne peut y avoir métamorphose à moins que le sujet n'ait d'abord séjourné à de basses températures pour des périodes dont la durée est liée aux conditions dans lesquelles s'est effectué le développement embryonnaire et larvaire.

Parce que des traitements qui se sont montrés efficaces pour d'autres insectes n'ont pu mettre fin au sommeil hivernal de *D. polytomum* après trois ou quatre mois, nous ne sommes donc pas justifiés de conclure que chez cette espèce la diapause est héréditaire. Ces résultats ne prouvent qu'une chose: c'est que la reprise des activités n'est pas influencée, autant qu'on pourrait le croire, par les conditions prévalant, soit durant l'hiver, soit au printemps, lors de la métamorphose.

Si l'on compare les conditions climatériques dans les régions d'une ou plusieurs générations, l'on constate plusieurs faits venant à l'appui de cette hypothèse. Suivant Balch (7), le pourcentage de larves qui se transforment en adultes chaque année est toujours plus petit à mesure que l'on se dirige vers le nord. Dans la Gaspésie, la moyenne d'émergence est de près de 20%, dans le centre du Nouveau-Brunswick elle est d'environ 70%, tandis qu'au sud de la Nouvelle-Angleterre, elle est encore beaucoup plus élevée. Serait-ce les conditions existant durant l'hiver ou le printemps qui pourraient être responsables des variations extérieures? Apparemment non, car durant tout son cycle vital c'est lorsque la larve est dans son cocon que les conditions de milieux se ressemblent le plus, indépendamment des régions ou des années. Ainsi, durant l'hiver, que ce soit dans la Gaspésie ou la Nouvelle-Angleterre, la température du sol sous la neige est à peu près la même. De plus, dans les peuplements d'épinette, où se trouve *D. polytomum*, certains sols peuvent être plus saturés les uns que les autres, mais au printemps, durant la période de nymphose, l'humidité en général est partout très élevée. Ce qui change le plus suivant les régions et les années, c'est la température de l'humus forestier après l'hibernation. Mais là encore, il est peu probable que les différences de température soient suffisamment élevées pour permettre d'expliquer les variations considérables de développement.

Dans les régions à une ou deux générations, ce n'est pas tant durant l'hibernation et la nymphose comme durant le développement embryonnaire et larvaire que l'on constate de grandes différences dans les conditions de milieu. Ainsi, dans certaines parties du comté de Rimouski et de la Gaspésie, il n'y a pas eu en 1937 et en 1938, un seul mois de l'année, où la température à 4 pieds du sol n'a pas descendu à 32°F. Il est peu probable que dans le sud du Nouveau-Brunswick et dans la Nouvelle-Angleterre l'on puisse observer en juillet et août de telles chutes thermiques aussi nettement défavorables aux œufs et aux larves et qui pourraient être suffisantes pour provoquer des diapauses de deux ou trois ans. En juin, alors que l'émergence bat son plein, il arrive souvent dans la Gaspésie et sur la côte nord du St-Laurent que nous ayons une suite de journées froides qui empêcheront toute activité chez les femelles récemment écloses. Il en résulte un stage prolongé des œufs dans l'organisme maternel qui, d'après Roubaud, occasionnerait une espèce d'intoxication se traduisant plus tard par de longues diapauses.

De l'ensemble des faits précités, il semble que si l'on veut avoir une explication des longues diapauses de *Diprion polytomum* dans la partie nord de son habitat, nos études devraient être concentrées beaucoup plus sur les facteurs agissant au cours du développement embryonnaire et larvaire, que sur les conditions de milieu lorsque la larve est dans son cocon, c'est-à-dire durant l'hibernation et la nymphose. De telles études ne peuvent être faites dans un milieu extérieur fluctuant. Elles doivent être exécutées en laboratoire, afin d'obtenir des stocks homogènes, élevés dans des conditions optima. De plus ces élevages doivent se poursuivre durant plusieurs générations, car même si la diapause n'est pas héréditaire, il n'en reste pas moins vrai qu'il

y a là, un rythme acquis depuis très longtemps et, comme l'a fait remarquer Cousin au sujet d'autres insectes, ce rythme peut fort bien ne pas disparaître complètement dès la première génération.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

IV. INFLUENCE OF FOOD AND DIAPAUSE UPON REPRODUCTIVE CAPACITY[1,2]

By M. L. Prebble[3]

Abstract

Methods of sampling, determination of reproductive capacity, and analysis of data are described. Various physical measurements are positively correlated with reproductive capacity, but regression equations are unsatisfactory for estimation of fecundity outside of the population in which the relationships have been determined, due to variability in the degree of joint variation of size and fecundity under different feeding conditions. Field populations developed on white spruce are more fecund than those developed on black spruce; reductions of 30% or more may result from periodic food shortage.

Reproductive capacity of females emerging over a period of three to five years in each of 20 populations, failed to show any consistent trend in relation to the diapause period. From this, and also from the slight reduction in eonymphal dry weight over extended intervals at favourable temperature, it is concluded that the destruction of eonymphal reserves during diapause proceeds very slowly and has no practical effect upon fecundity of females issuing after prolonged diapause.

The reproductive capacity of an insect may be influenced by quantity and quality of food during larval development, and by food, water, copulation, viability of introduced sperm, longevity, and population density, among factors affecting the adults (3, 5, 6, 7, 8, 11, pp. 391–394). The problem of determining the influence of factors during larval development may therefore be far from simple in species dependent upon a variety of factors during adult life. The problem in *Gilpinia polytoma* (Hartig) is less complicated because of several features: (1) absence of feeding after the fifth moult; (2) the parthenogenetic mode of reproduction; (3) the apparent lack of influence of environmental fluctuations during imaginal life upon ovarian development; and (4) the limited reproductive capacity which reduces the labour of counts.

Repeated tests have disclosed no consistent relationship between the following pairs of variables: (1) number of ova and percentage deposited; (2) adult longevity and number of ova; and (3) adult longevity and percentage of ova deposited. Females emerging after prolonged diapause are as vigorous as individuals without diapause. Percentage of hatch, determined in eight years in central Gaspé and two years in New Brunswick, has been uniformly

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[2] *Papers I and II of this series appeared in the October issue of the Canadian Journal of Research; Paper III in the November issue.*

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high, nearly always over 90%. For these several reasons, the influence of food and diapause upon reproductive capacity of the spruce sawfly may be restricted to a study of the number of oocytes capable of being developed to maturity, all environmental effects during adult life being extraneous to the problem. Although the investigations were concerned chiefly with the effects of diapause, larval food, as will appear shortly, cannot be ignored in comparative tests.

The basic consideration is that reserves laid up in the eonymph must both sustain the insect during diapause and subsequent development, and also serve for the nourishment of oocytes in the maturing insect. Metabolic demands during prolonged diapause, if sufficiently large, might therefore be expected to result in reduced reproductive capacity.

Methods of Determination

The individual reproductive capacity of approximately 9000 females was determined over a period of years. Some females were placed singly in small cages in the field or laboratory, and the number of deposited eggs and retained oocytes determined. The much simpler method of counting oocytes in newly emerged females, considered capable of being developed to mature ova, was used in most instances. The defence of results thus obtained rests on an argument involving description of ovaries at adult emergence, development of oocytes during adult life, and a comparison of statistics obtained by the two methods.

Description of Ovaries

Each ovary consists typically of 15 ovarioles of the polytrophic type, the greenish-white oocytes* being readily distinguishable from the mottled dark and white nurse cell follicles. Though Smith (10) found four to five small differentiated oocytes in sections of pronymphal ovarioles, the present author has never found so many as a typical number either in newly emerged females, or in others at death, in which oviposition had been prevented. The average for field and laboratory reared females is between one and two oocytes per ovariole; occasionally three occur in a few ovarioles, and in one rare instance a female developed 102 ova, an average of 3.4 per ovariole. Apparently two or three of the young oocytes recognized by Smith in pronymphal ovarioles are arrested in their development and possibly absorbed before yolk formation.

The oocyte nearest the oviduct is usually mature at adult emergence, and the accompanying nurse cells are exhausted. Oocytes toward the germarium are smaller with larger nurse cells. Occasionally adults emerge precociously with all oocytes very small and the abdomen distended with fat body. Sterile females, with no development of the ovaries, were rare in this study, and were not attributable to poor food conditions, since they occurred at a frequency of 11 : 442 in an incubator reared pure line as compared with a ratio of 9 : 8100 in field material, much of which matured under severe conditions of host defoliation. Though the ovarioles within one ovary may

* *Yellow in fixed material.*

be variably developed, there is usually a remarkably close similarity in the production of the two ovaries of a pair.

Development of Oocytes

Mature ova measure about 2.0 by 0.3 mm. Most of the smaller oocytes which at adult emergence have a clearly recognizable yolk vary from one-quarter to three-quarters the size of ova. Some, however, are only about 0.2 mm. long, yet with sufficient yolk to distinguish them from the nurse cells. The presence of yolk, and not the size, was used as a criterion to establish which of the small oocytes should be included in the counts.

The development during adult life of oocytes which were small at emergence has been demonstrated by analysis of samples at different times. Immature oocytes constituted from 15 to 30% of the total capacity in newly emerged females in different populations, while at death of females in equivalent samples they constituted 2 to 5% of the total capacity. It is shown later that there was no essential difference in the total capacity of females dissected at emergence and at death, hence the reduction in immature oocytes proves that many of them were brought to maturity during adult longevity. This was entirely independent of oviposition.

There was no correlation between the percentage of immature oocytes at adult emergence and the duration of diapause, or between the percentage of immature oocytes and total capacity. It follows that high capacity and low capacity females were not so called merely by virtue of incidentally different stages of ovarian development at time of emergence. This is most clearly shown by the data in Table I, in which the females in different sample series from several populations are divided into classes of reproductive capacity representing the full range of variation. The proportion of immature oocytes was relatively constant for the different classes within each series and was much reduced, as already indicated, in series of females dissected at death. The relative constancy of the proportion of immature oocytes at emergence indicates a balance between the stored reserves and the number of oocytes that undergo development to and beyond the stage of yolk formation. This is a fortunate circumstance, since if there were a partial development of many oocytes prior to emergence, with subsequent absorption during adult life, counts made at emergence would be very inaccurate as a measure of reproductive capacity.

Comparative Results by Two Methods

Statistics on the average reproductive capacity of eight populations, as determined from series of females taken at random for dissection at emergence, dissection after oviposition, or dissection at death when oviposition was prevented, are summarized in Table II.

The mean reproductive capacity ($\bar{x}$) as determined in the oviposition and longevity series was not significantly different from that based on the emergence series in eight of the nine comparisons. In the other comparison (Experiment 302), in which the sample sizes were large, the difference though small was

TABLE I

THE RELATIVE CONSTANCY OF IMMATURE OOCYTES THROUGHOUT THE ENTIRE RANGE OF REPRODUCTIVE CAPACITY, FOR SERIES OF FEMALES FROM DIFFERENT POPULATIONS. *N* IS THE NUMBER OF FEMALES FALLING WITHIN THE VARIOUS CLASSES OF REPRODUCTIVE CAPACITY; THE PERCENTAGE VALUE IS THE RELATION OF IMMATURE OOCYTES TO THE TOTAL NUMBER OF OOCYTES

Expt. No.	Series*	Class limits of reproductive capacity																Entire series	
		0 – 25		26 – 30		31 – 35		36 – 40		41 – 45		46 – 50		51 – 55		56 – 60			
		N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
123 (1937)	Em.	—	—	8	24.3	18	20.1	23	25.6	25	23.6	14	27.1	12	23.4	—	—	100	24.2
	Ov.	—	—	3	2.4	8	2.6	12	3.5	11	3.2	8	3.9	7	5.8	—	—	49	3.8
	Lon.	—	—	7	4.7	6	6.5	6	6.4	4	2.4	6	5.9	14	6.4	—	—	43	5.8
154 (1938)	Em.	24	26.6	29	27.8	62	22.1	59	22.9	52	25.1	24	25.5	17	21.2	—	—	267	24.0
	Ov.	5	4.3	10	3.5	17	3.6	16	4.7	11	3.6	3	2.8	3	4.7	—	—	65	3.9
302 (1938)	Em.	—	—	44	31.1	29	27.4	63	23.9	84	24.6	114	23.4	97	24.3	96	21.3	529	24.0
	Ov.	—	—	19	1.8	34	1.4	36	1.7	59	1.6	55	3.3	33	2.2	27	2.0	263	2.2

* Em. = *emergence series;* Ov. = *oviposition series;* Lon. = *longevity series.*

TABLE II

COMPARISON OF MEAN REPRODUCTIVE CAPACITY IN DIFFERENT SERIES OF FEMALES TAKEN AT RANDOM FROM A COMMON SOURCE

Expt. No.	Series	Number of females	$\bar{x}$	$S.E._{\bar{x}}$	Mean difference*	S.E. (diff.)	Significance
96 (1935)	Emergence	75	51.92	0.93			
	Oviposition	50	50.38	1.17	1.54	1.49	None
96 (1936)	Emergence	100	44.57	1.04			
	Oviposition	14	41.21	1.94	3.36	2.20	None
106 (1936)	Emergence	100	47.84	0.81			
	Oviposition	62	46.69	1.28	1.15	1.52	None
115 (1936)	Emergence	100	36.83	0.80			
	Oviposition	9	38.67	3.40	−1.84	3.49	None
98 (1937)	Emergence	200	36.24	0.59			
	Oviposition	22	34.36	1.34	1.88	1.46	None
123 (1937)	Emergence	100	40.83	0.83			
	Oviposition	49	41.61	1.08	−0.78	1.36	None
	Longevity**	43	42.84	1.83	−2.01	2.01	None
302 (1938)	Emergence	853	45.91	0.37			
	Oviposition	263	43.67	0.59	2.24	0.70	Good
154 (1938)	Emergence	268	37.09	0.53			
	Oviposition	65	35.95	1.07	1.14	1.19	None

* *The mean of the emergence series is the basis in all comparisons.*

** *Oviposition was prevented in the longevity series.*

statistically significant. From this, considering also that in the other comparisons there was a majority of cases in which the determination based on the emergence series was the higher, there is some evidence of absorption of a few small oocytes during adult life, and consequently, of a corresponding positive error in determinations of reproductive capacity based on dissections of females at emergence. However, the discrepancy was small and the values obtained were evidently close approximations of true reproductive capacity. Inaccuracies inherent in the technique affect in a similar way practically all the determinations used in analysis of the influence of food and diapause, and therefore cancel out in the comparisons.

Methods of Analysis

Nature of Frequency Distributions

The nature of the frequency distributions of females according to their reproductive capacity requires description for two reasons: (1) normality or near normality of the distributions is a prerequisite of the usual techniques of analysis; (2) only by being assured of the adequacy of these techniques to the data in hand is one apt to appreciate the causes operative in obscuring relationship between diapause and reproductive capacity.

Much speculation was aroused when the possibility of various modal points in the population, suggesting the existence of strains differing in reproductive capacity, was indicated by graphic analysis of some of the earlier sample series of females. To reach a judgment as to the significance of the apparent modal points, three lines of investigation were carried on, none of them being a direct attack (which could only be attained by a program of selective line rearings beyond the possibilities of this project) but in the aggregate leading to a fairly certain conclusion.

The first phase of the investigation consisted of drawing off, from a known normal population of 1000 cardboard squares, samples of 50, 100, and 200 units, each unit being replaced in the population after the draw. Fairly

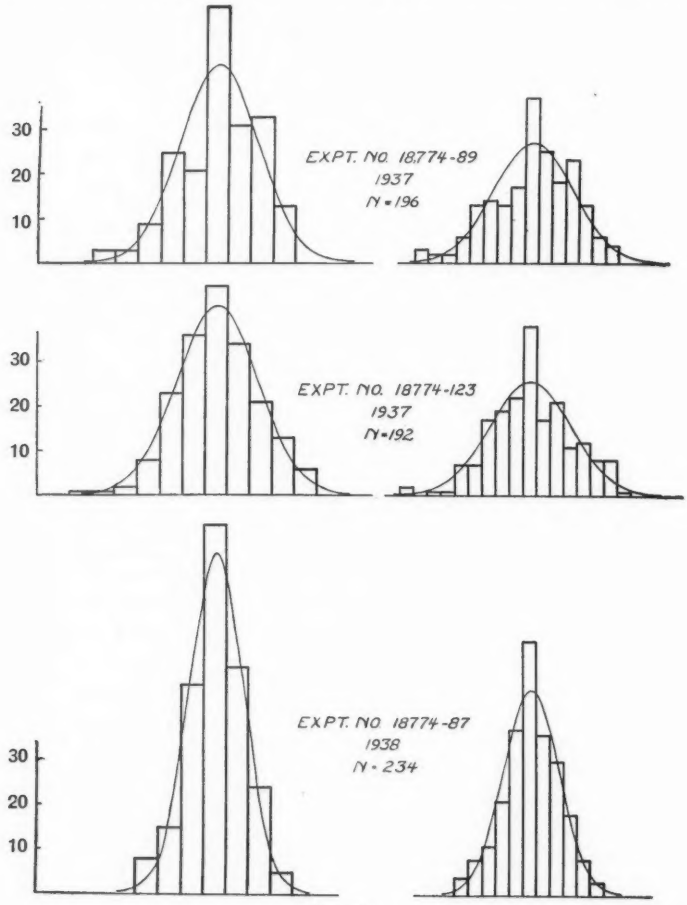

FIG. 1a

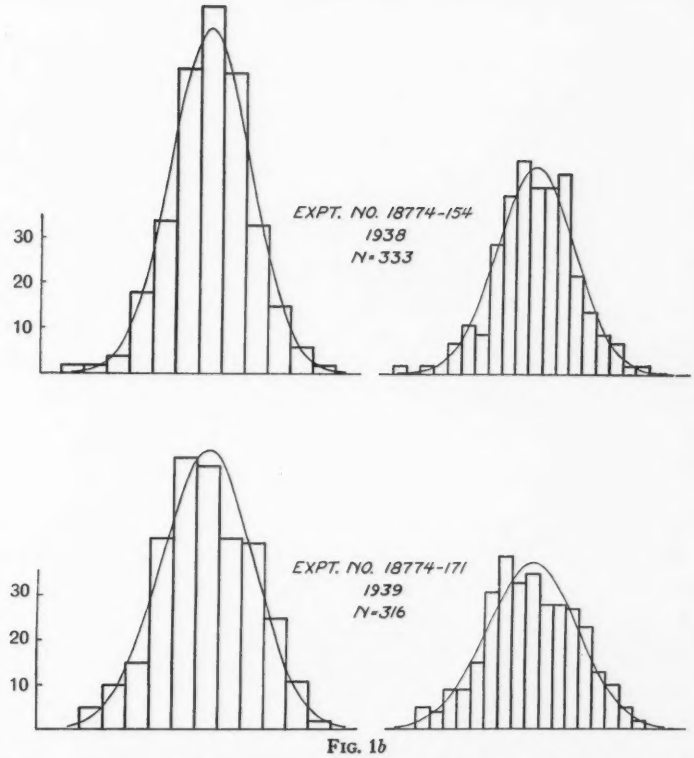

FIG. 1b

FIG. 1. *Histograms and the theoretical normal distributions for samples of spruce sawfly females classified by the number of developing oocytes in the ovaries at emergence. Curves at the left are based on groupings in classes of five oocytes, those at the right on groupings in classes of three oocytes: a, samples of about 200 females; b, samples of over 300 females.*

large departures from the population distribution occurred due to chance alone. Apparent modal points, having of course no real existence, were not uncommon in histograms based on samples of 50 and 100 units grouped in classes of three and five units, and occurred also in samples of 200 units. This showed conclusively that even with moderately large samples one must not expect distribution curves free of irregularity due to purely chance influences.

The later series of sawfly females were made as large as reasonably possible. The histograms for five series are illustrated in Figs. 1*a* and 1*b*; those at the left are based on groupings in classes of five oocytes, and those on the right, in classes of three oocytes, and the theoretical normal distributions are indicated by the smooth curves. The normality of the five distributions illustrated, and of seven others in which the sample size was moderately large, has been tested statistically by the Chi square test (Table III). The method

TABLE III

CHI SQUARE TESTS OF THE NORMALITY OF FREQUENCY DISTRIBUTIONS OF FEMALES CHARACTERIZED BY NUMBER OF OOCYTES. *P* INDICATES THE PROBABILITY THAT THE OBSERVED DISTRIBUTION CONFORMS TO THE HYPOTHESIS OF NORMALITY

Expt. No.	Number of females	Class interval, 3			Class interval, 5		
		Chi square	D.f.	*P*	Chi square	D.f.	*P*
25 (pure line)*	347	24.71	10	.01–	18.02	6	.01–
89 (1937)	196	15.41	10	.12	16.59	4	.01–
89 (1938)	345	17.86	10	.06	16.39	5	.01–
89 (all years)	662	22.39	13	.05	19.59	7	.01–
87 (1938)	233	7.19	6	.30	2.06	3	.56
87 (all years)	464	29.19	9	.01–	20.31	5	.01–
106 (1936)	162	6.56	9	.68	2.37	4	.67
98 (1937)	222	10.45	8	.24	5.41	4	.25
123 (1937)	192	12.91	10	.23	1.88	5	.86
154 (1938)	333	10.28	10	.42	3.83	6	.70
171 (1939)	316	9.76	12	.64	7.32	6	.29
13 (1939)*	233	14.74	10	.25	4.60	4	.34

* *New Brunswick material; all other lots were Gaspé material.*

need not be described here and it is only necessary to remark that the tests were very rigorous since three degrees of freedom were subtracted, because of the use of N, $\bar{x}$, and $S.E.$ in the calculation of expected frequencies.

Five of the distributions based on groupings in classes of five oocytes, where degrees of freedom were necessarily reduced in number, had significant departures from normality; on the basis of groupings in classes of three oocytes, only two distributions had significant departures. The failure of several distributions to conform to the hypothesis of normality was due to moderate skewness, but since the means of moderately skewed distributions tend toward normality, these may be analysed in the usual way.

The final phase of the investigation was the analysis of reproductive capacity of females in a pure line, all descended parthenogenetically from a single individual in incubator rearings at constant conditions of food and temperature. The distribution was not free of the apparent modal points, and had a significant departure from the corresponding normal distribution (P less than .01) due to positive skewness. These results are instructive, since they prove that significant departures are not to be identified with heterogeneity in the population due to strains differing in reproductive capacity.

These considerations led to the conclusion that the apparent modal points in several of the distributions were merely artifacts due to chance, and that the analysis of data by procedures drawn up for normal populations was fully justified.

Method of Sampling

The method of making up the samples needs to be considered. In some of the experimental populations, very large numbers of females emerged

TABLE IV

OOCYTE COUNTS OF FEMALES DISSECTED AT EMERGENCE, IN SUCCESSIVE SUBSAMPLES OF FEMALES DURING THE EXTENDED EMERGENCE PERIOD

Expt. No.		Successive subsamples						Entire series
		1	2	3	4	5	6	
29 (1935)	Period	July 10 – 14	July 15 – 18	July 19 – 21	July 22 – 25	—	—	
	N	29	19	16	26			90
	$\bar{x}$	36.07	35.84	41.06	42.54			38.79
	$S.E.\bar{x}$	0.95	2.10	1.25	1.84			0.84
96 (1936)	Period	July 6 – 7	July 8 – 9	July 10 – 12	July 13 – 15	July 16 – 22	—	
	N	20	25	17	18	20		100
	$\bar{x}$	45.90	44.56	46.94	41.39	44.10		44.57
	$S.E.\bar{x}$	2.09	2.32	2.31	2.53	2.36		1.04
106 (1936)	Period	July 6 – 7	July 8	July 9 – 10	July 11 – 14	July 16 – 20	—	
	N	15	23	25	21	16		100
	$\bar{x}$	49.87	49.65	45.56	47.33	47.56		47.84
	$S.E.\bar{x}$	2.16	1.62	1.39	1.93	2.29		0.81
89 (1937)	Period	July 1 – 6	July 8 – 11	July 12 – 16	July 17 – 19	July 20 – 30	—	
	N	40	38	42	32	44		196
	$\bar{x}$	39.20	36.42	38.64	44.12	39.48		39.41
	$S.E.\bar{x}$	1.39	1.31	1.50	1.22	1.28		0.63
98 (1937)	Period	July 1 – 5	July 6 – 8	July 9 – 11	July 12 – 14	July 16 – 17	July 18 – 20	
	N	29	38	35	35	36	27	200
	$\bar{x}$	36.83	39.79	33.69	33.20	38.33	35.04	36.24
	$S.E.\bar{x}$	1.30	1.13	1.07	1.62	1.37	1.88	0.59
154 (1938)	Period	June 17 – July 5	July 6 – 8	July 9	July 10 – 12	July 13 – 15	July 16 – Aug. 7	
	N	24	50	97	40	29	28	268
	$\bar{x}$	38.25	39.02	36.41	38.18	34.76	35.89	37.09
	$S.E.\bar{x}$	1.74	1.21	0.85	1.28	1.87	1.66	0.53
302 (incubator)	Period	Feb. 26 – Mar. 11	Mar. 12 – 17	Mar. 18 – 22	Mar. 23 – April 1	April 2 – 23	—	
	N	127	103	100	102	99		531
	$\bar{x}$	46.79	46.14	48.26	46.58	42.22		46.05
	$S.E.\bar{x}$	0.89	1.07	1.02	1.14	1.25		0.48

during three or four weeks. The earlier practice in such cases was to take the first 100 to 200 females as the sample. If high capacity and low capacity individuals tended to emerge at different times samples restricted to one portion of the emergence period might be seriously biased. In order to make an analysis of this point, the females in seven series were broken down into subsamples according to time of emergence (Table IV) and the subsample means critically compared (Table V).

Significant differences between the highest and lowest subsample means occurred in five of the seven series, but significant differences between the general sample mean (best estimate of population mean) and means of component subsamples occurred in only four of 36 comparisons. There was no evidence of a tendency for the more fecund and less fecund individuals to

TABLE V

ANALYSIS OF DIFFERENCES BETWEEN THE MEANS OF VARIOUS SUBSAMPLES OF FEMALES DISSECTED AT EMERGENCE. MARGINAL DIFFERENCES HAVE BEEN TESTED BY THE CALCULATION OF "t" AS DESCRIBED IN TEXT

Expt. No.	Highest and lowest subsample means			General mean and highest subsample mean			General mean and lowest subsample mean			Number of subsamples not significantly different from general mean	Position in series	
	Diff.	*S.E.*	Signif.	Diff.	*S.E.*	Signif.	Diff.	*S.E.*	Signif.		*(a)*, of lot with highest mean	*(b)*, of lot with lowest mean
29	6.70	2.79	Yes	3.75	2.02	Yes*	2.95	2.26	No	3 of 4	Latest	2nd of 4
96	5.55	3.43	No	2.37	2.53	No	3.18	2.74	No	5 of 5	3rd of 5	4th of 5
106	4.31	2.57	No	2.03	2.31	No	2.28	1.61	No	5 of 5	Earliest	3rd of 5
89	7.70	1.79	Yes	4.71	1.38	Yes	2.99	1.46	No*	4 of 5	4th of 5	2nd of 5
98	6.59	1.97	Yes	3.55	1.28	Yes	3.04	1.72	No	5 of 6	2nd of 6	4th of 6
154	4.26	2.23	Yes*	1.93	1.32	No	2.33	1.94	No	6 of 6	2nd of 6	5th of 6
302	6.04	1.61	Yes	2.21	1.13	No	3.83	1.34	Yes	4 of 5	3rd of 5	Latest

** The conclusion as to significance, contrary to the relationship of the values shown, is based on the more critical "t" test.*

emerge at restricted intervals in the emergence period (Table V, last two columns). Neither was there any significant difference in the degree of variability* among individuals emerging at different times. In conclusion, sampling restricted to a limited portion of the emergence period would only occasionally introduce a bias, and this possibility was eliminated in most of the experimental series that were studied either by including all emerging individuals in the sample, or by taking representatives over the entire emergence period.

Significance of Differences

In the calculation of the standard error of a mean difference between two series, the formula,

$$S.E.\ (\bar{x}_1 - \bar{x}_2) = \sqrt{(S.E.\ \bar{x}_1)^2 + (S.E.\ \bar{x}_2)^2}$$

** The coefficients of variability for the various subsamples have not been included in the already extensive tabulation, but are easily derivable from the statistics shown.*

was used as a first approximation (all standard errors of differences shown in the tables were so derived), in the test of significance of the mean difference; the latter having to be at least twice the standard error to be adjudged significant. This test, however, lacks precision, especially where samples being compared are not large and differ in size. Therefore all marginal cases were tested by a more rigorous test in which the two individual variances are pooled to provide the best estimate of the difference variance and standard error. The significance of the critical "t" value derived from the mean difference and its standard error also depends upon sample size. The method is fully described in standard texts (2, pp. 40–42) and needs no further comment here.

Food and Reproductive Capacity

Larvae of *Lymantria dispar* Linn. which were starved on alternate days during the final instar produced pupae 48% lighter in weight, and female adults 53% lower in egg production, than controls (4). The production of viable eggs by *Ephestia kuhniella* Zeller was 14 to 32% less with white flour as the larval food, compared with wholemeal, and 44% less when the larval ration of wholemeal was reduced by 50% from the optimal ration of 0.15 gm. per larva (7).

Reproductive capacity in the spruce sawfly is significantly correlated with various physical measurements. The results of some preliminary analyses in which the possibility of using some simple measurements as an index of reproductive capacity was investigated, are shown in Table VI. Gross

TABLE VI

CORRELATION BETWEEN REPRODUCTIVE CAPACITY AND PHYSICAL MEASUREMENTS IN VARIOUS SAWFLY POPULATIONS, AND THE REGRESSION EQUATIONS FOR ESTIMATING REPRODUCTIVE CAPACITY (Y) FROM THE MEASUREMENT (X)

Measurement	Source of material	Correlation coefficient	Regression equation
Head width*	New Brunswick field material, 123 females	$+.667 \pm .050$	$Y = -71.088 + 3.8534\ X$
Head width	Gaspé field material, 853 females	$+.526 \pm .025$	$Y = -130.528 + 5.481\ X$
Head width	New Brunswick pure line in incubator, 347 females	$+.652 \pm .031$	$Y = -147.810 + 6.3254\ X$
Gross weight, cocoon and contents (cg.)	New Brunswick pure line in incubator, 347 females	$+.758 \pm .023$	$Y = 2.968 + 7.861\ X$
Cocoon length (mm.)	New Brunswick pure line in incubator, 347 females	$+.652 \pm .031$	$Y = -61.712 + 13.231\ X$

* *Head width of adult in arbitrary units, 50X = 3.7084 mm.*

weight, just after cocoon spinning, was most highly correlated with reproductive capacity ($r = .758$); cocoon length and head width of the adult were slightly less valuable as indices of reproductive capacity. It is most instructive to note, that while a fair estimate of reproductive capacity within a given population could be obtained from the corresponding regression equation, the

latter was unsatisfactory for prediction outside of the population on which it was founded. This is evidenced by the dissimilarity of the three regression equations, reproductive capacity on head width, for different populations. This means that while size and fecundity vary in the same direction under the influence of food and other factors during larval development, the degree of their joint variation is not precisely the same in different populations. This recalls the experience of Schedl (9) who was in error by 43% of the actual fecundity in estimates of a Danzig population of *Diprion pini* Linn., based on the regression established for a population of the insect near the Dutch frontier.

White spruce as the larval host typically yields larger and more fecund females than black spruce (Table VII). The mean reproductive capacity

TABLE VII

COMPARISON OF THE MEAN REPRODUCTIVE CAPACITY OF FIELD COLLECTED MATERIAL FROM WHITE SPRUCE AND BLACK SPRUCE. ALL LOTS WERE FROM CENTRAL GASPÉ AND THE FEMALES EMERGENTS OF THE FIRST SEASON AFTER COCOON COLLECTION, EXCEPT WHERE OTHERWISE STATED

	Expt. No.	N	$\bar{x}$	$S.E._{\bar{x}}$	Remarks
Host White spruce					
1935	7	14	45.43	1.69	Emergents after 5-year diapause
	31	61	36.97	0.87	Emergents after 4-year diapause
	85	18	62.05	1.33	
	89	83	48.96	1.06	
	96	125	51.30	0.73	
1936	104	100	50.80	0.92	
	106	162	47.40	0.70	
1937	300	105	37.60	0.97	
	301	69	45.83	1.03	
1938	137	15	57.93	2.15	
	143	18	57.00	1.57	
1939	167	70	60.03	1.19	
	300 N	60	55.17	0.91	Material from York Co., N.B.
Host Black spruce					
1935	29	90	38.79	0.84	Emergents after 5-year diapause
	30	83	35.89	1.09	Emergents after 4-year diapause
	98	70	38.97	1.15	
1936	115	109	36.98	0.78	
1937	123	192	41.48	0.65	
	120	132	47.11	0.75	
1938	154	333	36.87	0.47	
	1	122	53.37	0.57	Material from Sunbury Co., N.B.
1939	165	122	45.66	0.81	
	171	316	39.02	0.57	
	13	233	53.00	0.55	Material from Sunbury Co., N.B.

of field populations on white spruce was occasionally over 60, frequently over 50, and lower values were due to unsatisfactory feeding conditions; on black spruce, a value of 50 to 55 indicated excellent feeding conditions, one less than 40, a scarcity of suitable foliage.

Experimental evidence of the effect upon fecundity of intermittent food shortage during late larval development was obtained by random division of field collected fourth instar larvae into two groups, one of which was continually supplied with foliage, and the other deprived of food for alternate intervals of two to three days. In three replications (Table VIII), the survivors of the second group suffered a reduction of 27 to 37% in reproductive capacity.

TABLE VIII

COMPARISON OF THE MEAN REPRODUCTIVE CAPACITY OF FEMALES ENSUING FROM LARVAE THAT WERE FED CONTINUALLY, AND FROM OTHERS INTERMITTENTLY STARVED DURING LATE LARVAL DEVELOPMENT. OTHER FACTORS WERE THE SAME FOR BOTH GROUPS, THE HOST WAS WHITE SPRUCE, AND THE REARINGS WERE CARRIED OUT IN CENTRAL GASPÉ

Replication	Basis	Larvae fed continually			Larvae intermittently starved			Reduction, %
		N	$\bar{x}$	*S.E.*$\bar{x}$	*N*	$\bar{x}$	*S.E.*$\bar{x}$	
1	Emergents of first year	68	47.75	0.76	82	33.85	0.63	29
(1934)	Emergents of all years	199	43.68	0.44	117	31.87	0.59	27
2	Emergents of first year	62	44.79	0.77	56	30.39	1.29	32
(1935)	Emergents of all years	124	43.67	0.55	126	27.71	0.88	37
3	Emergents of first year	20	37.65	1.10	13	24.46	1.74	35
(1935)	Emergents of all years	28	37.21	0.84	72	26.10	0.84	30

Studies of successive generations of pure lines reared without diapause in the incubator have shown that there is not necessarily any correlation between fecundity of parent and offspring, since this is determined within the limits of normal variation by the feeding regimen.

Diapause and Reproductive Capacity

The effect of diapause upon reproductive capacity appears to have received little study. The sole reference known to the writer is that of Cousin (1), who, as a result of observations and one experiment in which 25 normal females produced 10,619 maggot offspring and 25 diapause females produced 7591, concluded that diapause is prejudicial to fecundity of *Lucilia sericata* Meig. To what extent the reduced productivity was influenced by vigour, longevity, mating, hatching, and chance, was not determined.

The reproductive capacity of four series of sawfly females which emerged after four to five years in diapause is shown in Table VII. Comparison of these values among themselves, and with the values of other series lacking prolonged diapause, shows the futility of attempting to trace a relationship

between diapause and fecundity in different populations where uncontrolled factors, chiefly food, are so variable.

The procedure followed from 1935 onwards consisted of determining reproductive capacity of females emerging in successive years from a common source, by which is meant a large population of field collected larvae or cocoons from a single host and restricted area. In order for the successive yearly means to truly represent the influence of diapause, three basic conditions must be satisfied. 1. The original population must be homogeneous to keep experimental error as small as possible; this was controlled by limiting each population to material from one host, one locality generally less than an acre in extent, and one time of collection; tests of normality of distributions described previously provide evidence of homogeneity. 2. There must be no tendency for selective mortality among more or less fecund members of the population during diapause, a prerequisite for which no direct proof was obtained, but which seems to be established by presumptive evidence; if there were such selective mortality, females emerging in successive years would tend definitely towards greater or less fecundity, and the absence of any such inclination in 20 populations may be taken to substantiate the prerequisite. 3. The females emerging each year must provide a representative cross section of the then existing population, more and less fecund members in proportion to their occurrence in the population. Consideration of this prerequisite is undertaken in a later paragraph.

The data for several series of females from each of 20 populations are summarized in Table IX, and the intrapopulation differences are analysed in Table X. In 19 comparisons between first and second year series, 14 dif-

TABLE IX

MEAN REPRODUCTIVE CAPACITY ($\bar{x}$) OF FEMALES EMERGING IN SUCCESSIVE YEARS FROM A COMMON SOURCE

Expt. No.		1935	1936	1937	1938	1939
85	N	18	3	23	8	—
	$\bar{x}$	62.05	50.33	50.70	52.00	
	$S.E.\bar{x}$	1.33	6.64	1.32	1.91	
87	N	72	4	154	233	—
	$\bar{x}$	52.51	44.50	47.14	48.36	
	$S.E.\bar{x}$	0.99	3.28	0.61	0.40	
89	N	83	33	196	345	—
	$\bar{x}$	48.96	34.36	39.41	37.54	
	$S.E.\bar{x}$	1.06	2.05	0.63	0.43	
96	N	125	114	—	—	—
	$\bar{x}$	51.30	44.16			
	$S.E.\bar{x}$	0.73	0.95			
98	N	70	98	222	—	—
	$\bar{x}$	38.97	29.58	36.05		
	$S.E.\bar{x}$	1.15	1.04	0.55		

TABLE IX—*Concluded*

MEAN REPRODUCTIVE CAPACITY ($\bar{x}$) OF FEMALES EMERGING IN SUCCESSIVE YEARS FROM A COMMON SOURCE—*Concluded*

Expt. No.		1935	1936	1937	1938	1939
234	N	68	3	62	64	—
	$\bar{x}$	47.75	41.67	42.50	40.91	
	$S.E._{\bar{x}}$	0.76	0.68	0.65	0.57	
235	N	82	5	30	—	—
	$\bar{x}$	33.85	24.40	27.70		
	$S.E._{\bar{x}}$	0.63	3.15	0.92		
100	N	—	20	—	20	53
	$\bar{x}$		50.40		43.35	48.70
	$S.E._{\bar{x}}$		2.19		1.75	1.00
102	N	—	26	6	91	18
	$\bar{x}$		49.23	51.67	47.08	45.56
	$S.E._{\bar{x}}$		1.78	4.04	0.94	2.22
114	N	—	43	61	28	—
	$\bar{x}$		49.12	43.89	44.50	
	$S.E._{\bar{x}}$		1.42	0.94	1.52	
115	N	—	109	137	124	36
	$\bar{x}$		36.98	39.54	35.42	41.11
	$S.E._{\bar{x}}$		0.78	0.80	0.74	1.39
257	N	—	62	3	28	31
	$\bar{x}$		44.79	37.67	41.93	43.58
	$S.E._{\bar{x}}$		0.77	5.81	0.89	1.14
258	N	—	56	9	30	31
	$\bar{x}$		30.39	16.67	25.10	28.61
	$S.E._{\bar{x}}$		1.29	2.64	1.33	1.89
260	N	—	13	14	34	11
	$\bar{x}$		24.46	27.64	25.06	29.27
	$S.E._{\bar{x}}$		1.74	2.48	1.16	1.66
118	N	—	—	79	15	13
	$\bar{x}$			45.19	35.80	40.08
	$S.E._{\bar{x}}$			1.09	2.15	1.64
119	N	—	—	52	28	153
	$\bar{x}$			38.96	41.61	46.86
	$S.E._{\bar{x}}$			1.64	1.98	0.88
120	N	—	—	132	88	42
	$\bar{x}$			47.11	45.36	44.38
	$S.E._{\bar{x}}$			0.75	0.86	1.33
121	N	—	—	83	5	55
	$\bar{x}$			43.88	45.20	37.75
	$S.E._{\bar{x}}$			0.99	3.86	1.15
123	N	—	—	192	74	103
	$\bar{x}$			41.48	36.62	41.20
	$S.E._{\bar{x}}$			0.65	0.91	0.88
124	N	—	—	35	18	32
	$\bar{x}$			44.26	38.39	41.66
	$S.E._{\bar{x}}$			1.37	1.42	1.19

TABLE X

ANALYSIS OF DIFFERENCES BETWEEN MEAN REPRODUCTIVE CAPACITY OF FEMALES EMERGING IN DIFFERENT YEARS FROM A COMMON SOURCE. THE FIRST YEAR, ETC., REPRESENTS THE FIRST SEASON OF EMERGENCE AND WAS NOT NECESSARILY THE SAME CALENDAR YEAR IN DIFFERENT POPULATIONS. DIFFERENCES ARE POSITIVE REPRESENTING AN APPARENT INCREASE, EXCEPT WHERE NOTED TO THE CONTRARY

Expt. No.	1st to 2nd years			1st to 3rd years			2nd to 3rd years			3rd to 4th years		
	Diff.	*S.E.*	Signif.	Diff.	*S.E.*	Signif.	Diff.	*S.E.*	Signif.	Diff.	*S.E.*	Signif.
85	−11.72	6.77	No	−11.35	1.87	Yes	0.37	6.77	No	1.30	2.32	No
87	− 8.01	3.43	No*	− 5.37	1.16	Yes	2.64	3.34	No	1.22	0.73	No
89	−14.60	2.31	Yes	− 9.55	1.23	Yes	5.05	2.15	Yes	−1.87	0.76	Yes
96	− 7.14	1.20	Yes	—			—			—		
98	− 9.39	1.55	Yes	− 2.92	1.28	Yes	6.47	1.18	Yes	—		
234	− 6.08	1.02	No*	− 5.25	1.00	Yes	0.83	0.94	No	−1.59	0.86	No
235	− 9.45	3.21	Yes	− 6.16	1.12	Yes	3.30	3.28	No	—		
100	—			− 7.05	2.80	Yes	—			5.35	2.02	Yes
102	2.44	4.41	No	− 2.15	2.01	No	−4.59	4.15	No	−1.52	2.41	No.
114	− 5.23	1.70	Yes	− 4.62	2.08	Yes	0.61	1.79	No	—		
115	2.56	1.12	Yes	− 1.56	1.08	No	−4.12	1.09	Yes	5.69	1.58	Yes
257	− 7.12	5.86	No	− 2.86	1.18	Yes	4.26	5.88	No	1.65	1.45	No
258	−13.72	2.94	Yes	− 5.29	1.85	Yes	8.43	2.96	Yes	3.51	2.31	No
260	3.18	3.03	No	0.60	2.09	No	−2.58	2.74	No	4.21	2.03	No*
118	− 9.39	2.41	Yes	− 5.11	1.97	No*	4.28	2.70	No	—		
119	2.65	2.57	No	7.90	1.86	Yes	5.25	2.17	Yes	—		
120	− 1.75	1.14	No	− 2.73	1.53	No	−0.98	1.59	No	—		
121	1.32	3.98	No	− 6.13	1.52	Yes	−7.45	4.03	No	—		
123	− 4.86	1.12	Yes	− 0.28	1.09	No	4.58	1.27	Yes	—		
124	− 5.87	1.98	Yes	− 2.60	1.82	No	3.27	1.85	No	—		

* *Decision based on "t" test as described in text.*

ferences are negative, representing a decrease in fecundity, of which five differences are statistically significant; of the five positive differences, one is significant. In comparisons between first and third year series, 17 differences are negative (11 significant) and two differences are positive (one significant). In comparisons between second and third year series, and between third and fourth year series, positive differences are more frequent than negative ones, and seven of the 20 positive differences are significant. In several of the populations, females of the fourth year had approximately the same, or greater, mean reproductive capacity than females of the first year. The data provide little concrete evidence that females emerging in successive years have a progressively reduced fecundity due to the metabolic demands upon eonymphal reserves during diapause.

All steps in the procedures have been examined and found to be essentially sound, with the exception of the third prerequisite, viz., that each year's emergence must represent a true cross section of the then existing population. If this were true, and if metabolic demands during diapause were sufficiently great, then the distribution of females in different classes within the range of variation in reproductive capacity would be essentially the same from year to year, except for a gradual shifting toward the lower categories with prolongation of diapause. Comparisons of the distributions of females in

TABLE XI

DISTRIBUTION OF FEMALES IN DIFFERENT CLASSES WITHIN THE RANGE OF VARIATION IN REPRODUCTIVE CAPACITY, FOR EMERGENTS OF DIFFERENT YEARS FROM A COMMON SOURCE. THE VALUES SHOWN ARE THE PERCENTAGES OF THE TOTAL SEASONAL EMERGENCE FALLING WITHIN THE RESPECTIVE CLASSES

Expt. No.	Year	*N*	Class limits of reproductive capacity					
			0 – 30	31 – 35	36 – 40	41 – 45	46 – 50	51 –
98	1935	70	18.6	20.0	12.8	27.2	11.4	10.0
	1936	98	57.2	14.3	16.3	4.1	6.1	2.0
	1937	222	26.2	23.5	25.3	13.1	6.3	5.8
	All years	390	32.5	20.6	20.8	13.3	7.2	5.6
115	1936	109	22.0	22.9	24.8	15.6	9.2	5.5
	1937	137	14.6	14.6	24.8	19.0	13.1	13.9
	1938	124	24.2	26.6	23.4	13.7	8.9	3.2
	1939	36	11.1	13.9	13.9	33.3	11.1	16.7
	All years	406	19.2	20.5	23.4	17.7	10.6	8.6
120	1937	132	3.8	6.8	7.6	25.0	20.4	36.4
	1938	88	4.5	9.1	10.2	19.3	23.9	33.0
	1939	42	11.9	2.4	11.9	26.2	21.4	26.2
	All years	262	5.3	6.9	9.2	23.3	21.8	33.5
123	1937	192	9.4	16.7	21.3	20.8	14.6	17.2
	1938	74	23.0	17.6	27.0	21.6	6.8	4.0
	1939	103	11.7	13.6	18.4	21.4	22.3	12.6
	All years	369	12.7	16.0	21.7	21.2	15.2	13.2

successive years, for four populations with moderately large numbers, are shown in Table XI.

Only in one population, viz., 120, do the successive yearly distributions even moderately conform to the hypothesis. This, in fact, was the only one of the 20 populations studied over a period of years that showed a progressive reduction in reproductive capacity, though even here the differences were not significant.

The failure of the third prerequisite provides an explanation of the irregular fluctuations in annual mean reproductive capacity. Observed differences need be significant of nothing more than a departure in the distribution of seasonal emergents from the true population distribution. In fact, there is some evidence that the resumption of development may not affect at random the more and less fecund members of the population, but may be biased toward one or the other group according to wholly unknown factors. This is illustrated by the data in the accompanying synopsis, for various samples of a large cocoon population incubated under different conditions. The means of three samples were significantly different from the combined mean of all five (best estimate of the population mean), showing that even when difference in time was eliminated, the resumption of development was not purely at random among the members of the various samples, which incidentally were all large.

Conditions of incubation	Development, %	Number of females	$\bar{x}$	$S.E.\bar{x}$
1. Dec. 17: 74° F., 100% R.H.*	8–10	92	41.82	1.08
2. Feb. 7: Provided with contact water, then 74° F., 100% R.H.	60–95	531	46.05	0.48
3. Feb. 7: Dried two weeks, then contact water, and later 74° F., 100% R.H.	10	147	48.42	0.72
4. Feb. 7: Dried four weeks, then as in No. 3	7	74	46.55	1.20
5. Feb. 7: Dried six weeks, then as in No. 3	5	9	33.55	3.28
Combined results		853	45.91	0.37

* *R.H. = relative humidity.*

In conclusion, it would seem that prolonged diapause cannot entail a serious depletion of the eonymphal reserves, otherwise the ultimate effect upon reproductive capacity would not be masked by sources of error such as those described.

Changes in Weight During Diapause

In order to obtain more direct evidence of the rate of depletion of reserves, dry weight determinations** were made for samples of eonymphs in diapause in a number of experimental populations. Most of the samples consisted of 40 to 50 eonymphs. The results are briefly discussed in the following paragraphs.

a. Gaspé material, 1937–1938.

The average dry weight of eonymphs in diapause after 40 days at 75° F. was 1.96 cg., compared with an average of 1.98 cg. for eonymphs which, extracted from the cocoons at the start of the experiment, died within a few days. The difference is only 1.1 times its standard error and therefore not significant.

b. Gaspé material, 1938–1939.

The average dry weight of eonymphs after three months at 55° to 65° was 1.916 cg. (average of eight samples), and that of eonymphs killed at the start, 1.963 (average of 12 samples). The difference, 0.047 cg. with a standard error of 0.015, is statistically significant and represents a loss of about 2.4%.

** *Fat constitutes about 35% of the dry weight.*

c. New Brunswick material, 1938–1939.

Samples of cocoons were incubated at 74° F. Dry weight determinations were made at the start and after 10 days of incubation, 44 samples in all being analysed. The average weights were 2.19 cg. and 2.18 cg., the apparent loss (0.01 cg.) being less than its standard error (0.012) and without significance.

d. New Brunswick material, 1938–1939.

In another experiment with the same population as in *c*, dry weight determinations were made for various samples at intervals up to 18 days after incubation at 74° F. The accompanying synopsis is based on three complete replications involving 48 samples.

Period at 74°	Av. dry weight	Period at 74°	Av. dry weight
0	2.20	10	2.19
2	2.20	14	2.19
4	2.18	18	2.18
6	2.19		

In this series regression of dry weight on time, to a limit of 18 days, is without significance.

e. New Brunswick material, 1938–1939.

Dry weight determinations were made for successive samples from the same field population at intervals through practically a year, with results as follows.

Date	Av. dry weight	Date	Av. dry weight
Oct. 29, 1938	2.17	Mar. 7, 1939	2.26
Nov. 19	2.24	Mar. 21	2.21
Nov. 21	2.23	April 4	2.18
Dec. 6	2.21	April 7	2.19
Dec. 13	2.18	May 5	2.16
Dec. 27	2.20	May 13	2.18
Jan. 10, 1939	2.20	May 26	2.15
Feb. 6	2.18	Sept. 1	2.12
Feb. 21	2.16	Oct. 10	2.20
Feb. 22	2.22		

The regression of dry weight on time for this series indicates an apparent loss of about 2% during the year. The error of estimate is high, however, and it is informative to note that the final sample had a higher value than the first one.

With such slight weight changes during diapause it is not surprising that no clear trends in reproductive capacity with prolongation of diapause have been discovered.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

V. DIAPAUSE IN RELATION TO EPIDEMIOLOGY[1, 2]

By M. L. Prebble[3]

Abstract

This final paper in the series on diapause in the spruce sawfly describes the direct and indirect influences of diapause on epidemiology in different parts of the distribution range in eastern North America. These relate to duration of diapause, degree of diapause in overwintered cocoons, and proportions of the population surviving to participate in the continuation of the infestation. The nature of infestations in different areas is described briefly.

Discussion of features of intraspecific differences, and a summary of conclusions derived from all five papers in the series, are also included.

The degree of diapause in overwintered cocoons, the number of years that the insect may remain in diapause, the ultimate survival, and the number of seasonal generations, are variable factors in different parts of the distribution range of the spruce sawfly in North America and greatly influence the course of infestations. Reproduction by parthenogenesis is highly favourable to the insect, but this factor, being uniform throughout the distribution range, does not enter into comparisons between localities.

Diapause in a One-Generation Area

Duration of Diapause

Sawfly emergence in central Gaspé has been recorded in successive years for over 200 lots of field collected cocoons, which contained a mixture of generations of preceding years, and of reared cocoons of a single season. Each lot was kept in a shallow flat of wood and wire screening under the moss from September to early June, and in a wooden container with screen bottom resting on the forest floor in a shaded location, during the summer. Results for representative lots are summarized in Table I.

Emergence from a lot was seldom complete in less than four years, and frequently extended into the fifth or sixth year. There was a small emergence in the seventh year after spinning of the cocoons in a few large lots in which mortality during diapause was very low. Prolonged diapause was equally typical of mixed field populations, lots of cocoons spun at the same time and of the progeny of single females, regardless of the parental diapause period.

[1] *Manuscript received in original form March 29, 1941, and as revised, July 25, 1941. This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.*

[2] *Papers I and II of this series appeared in the October issue of the Canadian Journal of Research, Paper III in the November issue, and Paper IV in this issue.*

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TABLE I

EMERGENCE IN SUCCESSIVE YEARS FROM REPRESENTATIVE LOTS OF GASPÉ COCOONS. THE LOTS CONSISTED OF FIELD COLLECTED COCOONS UNLESS OTHERWISE NOTED

Expt. No.	Time of collection	Original number of cocoons	Emergence in successive years									Total emergence, %
			1932	1933	1934	1935	1936	1937	1938	1939	1940	
13	June, 1932	866	104	10	338	92	5	—	—	—	—	63
14	June, 1932	497	76	65	146	27	—	—	—	—	—	63
30	July 5, 1932	444	40	16	74	110	22	—	—	—	—	59
31	July 4, 1932	388	49	38	50	65	4	1	—	—	—	53
42	June, 1933	3322	—	1548	302	244	81	—	—	—	—	65
45	June, 1933	1519	—	725	21	189	163	3	—	—	—	72
46	June, 1933	435	—	99	5	9	7	1	—	—	—	28
49*	Sept. 1– 8, 1933	533	—	—	40	2	54	114	13	—	—	42
50*	Sept. 8–16, 1933	246	—	—	36	1	75	56	—	—	—	68
51*	Sept. 16–23, 1933	321	—	—	80	2	25	67	1	—	—	55
52*	Sept. 23–30, 1933	389	—	—	202	4	44	29	—	—	—	72
53*	Sept. 30–Oct. 7, 1933	273	—	—	186	0	5	1	—	—	—	70
63	June, 1934	2089	—	—	442	35	174	245	—	—	—	43
64	June, 1934	1840	—	—	410	33	192	309	10	—	—	52
65	June, 1934	1687	—	—	382	33	84	32	—	—	—	31
66	June, 1934	1696	—	—	387	8	188	65	2	—	—	38
81	June, 1934	494	—	—	8	1	74	244	21	—	—	70
85*	Aug. 20–Sept. 3, 1934	100	—	—	—	18	3	24	9	—	—	54
86*	Sept. 3–10, 1934	687	—	—	—	52	4	113	53	—	—	32
87*	Sept. 10–17, 1934	1303	—	—	—	75	4	188	238	—	—	39
88*	Sept. 17–24, 1934	1226	—	—	—	123	4	263	135	—	—	43
89*	Sept. 24–Oct. 1, 1934	1261	—	—	—	146	33	263	363	10	—	65
*A***	1934	199	—	—	—	84	6	30	18	4	—	71
*B***	1934	55	—	—	—	25	1	6	5	—	—	67
*C***	1934	232	—	—	—	115	14	32	29	—	—	82
*D***	1934	94	—	—	—	79	—	—	—	—	—	84
97	June, 1935	2259	—	—	—	506	284	434	3	—	—	54
98	June, 1935	3359	—	—	—	579	115	309	—	—	—	30
115	June, 1936	1367	—	—	—	—	364	142	125	42	6	50
116	June, 1936	1217	—	—	—	—	350	94	124	72	6	53
123	June, 1937	1072	—	—	—	—	—	520	77	104	10	66
125	June, 1937	857	—	—	—	—	—	364	104	152	0	†
126	June, 1937	659	—	—	—	—	—	246	47	78	0	56
152	June, 1938	712	—	—	—	—	—	—	160	17	52	†
153	June, 1938	616	—	—	—	—	—	—	112	15	21	†
154	June, 1938	1319	—	—	—	—	—	—	328	23	245	†

** Lots consisted of cocoons spun by field collected mature larvae, over intervals noted.*

*** Lots consisted of progeny of virgin females, reared in small cages. Parents of Lot A had a 1-year diapause period; of Lot B, a 2-year diapause period; of Lot C, a 3-year diapause period; of Lot D, a 4-year diapause period.*

† Lots contained living cocoons in the fall of 1940, to yield further emergence in 1941 and later.

Reduced emergence in the second year, followed by an increase in the third or fourth year, was typical of the great majority of lots under study. The tendency was independent of the calendar year in which the lots were started, so that the "second year low" in many lots coincided with the "third or fourth year high" in others; hence the trend could not have been due to climatic variations in different seasons. The trend was also observed in a number of experiments in which the cocoons were left in the undisturbed forest floor in which they were spun, eliminating the possibility that the unusual distribution of seasonal emergence was in some obscure way brought about by the method of handling the experimental lots. No explanation can be offered for the phenomenon.

A picture of the average trend of yearly emergence in the Gaspé population is given in the following synopsis, based on 134 lots started in various years and in which emergence was completed by 1940.

Year	1	2	3	4	5	6	Total
Number of sawflies	16,190	2862	7812	6103	295	14	33,276
Percentage of total	48.65	8.60	23.48	18.34	0.88	0.04	99.99

The total emergence over all years was close to 50% of the original number of cocoons. About half of this emergence occurred in the first year, and most of the balance in the third and fourth years.

Proof that prolonged diapause is an entirely normal phenomenon in central Gaspé was obtained from quadrates of the undisturbed forest floor, which were isolated by means of screen cages permanently staked down with the sides banked with moss to prevent entry of larvae seeking a place to spin. The yearly emergence from cages (each two feet square) in the black spruce slope, where initial cocoon population was dense, follows:

	1932	1933	1934	1935	1936	1937	1938	1939
Three cages set down in June, 1932	19	72	20	12	10	4	2	—
Six cages set down in June, 1934	—	—	108	32	53	35	13	2

Variations in Diapause

Representative data on variations in the degree of diapause in central Gaspé, based on cocoon samples collected in the spring before emergence started, are summarized in Table II. The validity of the estimates may be recognized from the close agreement of results obtained by different methods.

TABLE II

VARIATIONS IN THE PERCENTAGE OF DIAPAUSE IN OVERWINTERED COCOONS IN DIFFERENT FOREST TYPES IN CENTRAL GASPÉ, 1932–1940. ALL LOTS CONSISTED OF COCOONS COLLECTED IN THE LATE SPRING OF THE YEAR FOR WHICH THE RECORD IS GIVEN

Year	Berry Mountain Brook						Brandy Brook			
	White spruce flat		Black spruce slope		Black spruce flat		Black spruce slope		Black spruce flat	
	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %
1932	1736 1735*	95 96	1806 941*	88 89	268	91	—	—	—	—
1933	434	77	4841	53	—	—	—	—	—	—
1934	2499 9243†	77 74	6110	78	686	91	—	—	—	—
1935	2508 378*	86 82	2259 139*	78 73	—	—	3359 331*	83 88	—	—
1936	1414	88	1717	71	1142	69	2584	72	—	—
1937	33	76	219	53	512	67	2588	56	229	76
1938	522	92	419	81	2258	91	2647	77	2099	93
1939	511	78	526	74	1102 1070††	80 77	1450	72	941 7999† 1012††	83 82 78
1940	—	—	—	—	—	—	339 235* 461††	29 27 39	214* 467††	38 44

* *Analyses of cocoons collected periodically during pronymphal and pupal development.*

† *Periodic analyses of samples from a single large collection in early June.*

†† *Cocoons left in wire flats in the moss, analysed in the autumn. All other lots were kept in wooden containers in the moss.*

Diapause in the overwintered populations of different forests and years varied from about 30 to over 95%. There was only an approximate parallelism in the trend of the degree of diapause in contiguous areas from year to year, showing that other factors than climatic variations were involved. Studies already described have shown that temperature variations within the limits of occurrence in the Gaspé forest had no perceptible effect on diapause in the overwintered populations; and while moisture deficiency in late May and June in some instances notably increased the percentage of diapause, the extreme alternative, viz., an abundance* of moisture at all times, did not result in a considerable reduction in the phenomenon.

* *Moisture from melting snow and rainfall is usually abundant due to the water-retaining capacity of the deep moss in the Gaspé forest. Rainfall usually exceeds three or four inches per month during the growing season. Moss samples taken periodically in four seasons had an average water content of 320% (dry weight basis), and the underlying humus of 350%. The values never fell below 40 and 100%, respectively, in the driest periods.*

A fairly striking relationship between the time of larval maturity and the degree of emergence in the following season, later members of the population giving a significantly higher emergence than earlier members, occurred in several series in which the successive lots of larvae matured under progressively declining temperature conditions (cf. Lots 49 to 53, Table I). On the other hand, a progressive increase in emergence did not occur in other series in which the successive lots of larvae matured in the absence of a definite temperature decline (cf. Lots 85 to 89, Table I).

Another instance in which seasonal development was to a large extent conditioned in the previous autumn was noted in 1939 and 1940. An unusually large proportion (37%) of the overwintering population in the black spruce flat, Brandy Brook, consisted of very young to moderately developed pronymphs in the fall of 1939, a phenomenon which was not explained satisfactorily, but which nevertheless was largely contributory to the unusually low diapause (about 42%) in the same population in the 1940 season, since as explained elsewhere, individuals that enter the developmental season as pronymphs do not fail to develop.

Considering the peculiar trend of seasonal emergence in the experimental lots, it is logical to suppose that the degree of diapause in a natural population will be influenced by the relative importance of the component parts. A high proportion of two-year–old cocoons would presumably lower the general development very appreciably. The age composition of a natural population of sawfly cocoons is indeterminable, but definitely varies from year to year and reflects the relative success of the immediately preceding larval generation.

In conclusion, variations in the degree of diapause in central Gaspé cannot be explained satisfactorily on the basis of climatic variations. Much of the variability between populations of different stands and years is apparently due to the previous history of the populations, while the more or less uniformly high degree of diapause appears to be an inherent characteristic of the Gaspé population as a whole.

Epidemiology

The sawfly outbreak in central Gaspé was already in an advanced stage when discovered in 1930. In addition to advantages resulting from the mode of reproduction and absence of parasitism, the great extent of spruce forest provided an unusual opportunity for the development of a large and destructive population. As the studies of the bionomics continued it became evident that the course of the outbreak was largely influenced by the diapause phenomenon, which restricted the population of feeding larvae and exposed the dormant population to the continual operation of factors causing mortality.

The progress of the cocoon population in typical spruce stands was followed from year to year, and representative data for three stands appear in Table III. It should be noted that the cocoon shells are very durable and persist for many years, and that although the number of living cocoons varies from

TABLE III

COCOON POPULATION AND MORTALITY, SAMPLE PLOTS IN CENTRAL GASPÉ, COUNTS MADE IN JUNE

Locality	Year	Square feet in sample	Cocoons per square foot						Total mortality, %
			Living	Emerged	Chewed	Insect-killed	Dead	Total	
Berry Mt. Brook, white spruce flat	1933	80	6.0	3.0	15.7	1.4	11.0	37.1	76
	1934	96	20.3	4.3	17.2	1.2	9.3	52.3	53
	1937	34	2.0	9.7	24.6	8.1	12.9	57.3	80
	1937	35*	0.9	9.2	24.3	9.1	12.9	56.4	82
Berry Mt. Brook, black spruce slope	1932	100	14.4	11.5	16.6	3.5	1.5	47.5	45
	1933	600	8.7	10.4	16.8	3.4	2.3	41.6	54
	1934	288	22.2	10.1	20.7	2.4	1.8	57.2	43
	1935	224	11.0	11.9	29.9	2.5	1.9	57.2	60
Brandy Brook, black spruce slope	1935	200	17.6	12.8	20.8	5.2	4.0	60.4	50
	1936	200	13.5	15.4	22.8	4.9	5.2	61.8	53
	1937	312	9.3	20.1	31.3	5.7	5.0	71.4	59
	1938	200	13.9	27.4	43.7	7.7	5.8	98.5	58
	1939	200	8.6	30.0	45.9	11.2	5.4	101.2	62
	1940	200	5.3	31.2	48.2	12.6	4.8	102.1	64

* *Samples taken under trees that had been killed by defoliation in preceding years.*

year to year, the total number cannot show a decrease except as influenced by sampling fluctuations.

The principal causes of mortality were shrews, mice, and squirrels, which made characteristic chewed openings in the cocoons, elaterid larvae, which made small circular or irregular openings, and physical factors, especially excessive moisture, which caused the death of the insect without immediate change in the cocoon, although the latter tend to collapse later. The total mortality in active infestations ranged from about 45 to 65% in well drained black spruce slopes, and from about 50 to 75% in valley-bottom stands of white spruce.

It is obvious that mortality values based on the total number of cocoons have to be interpreted according to the proportion of living cocoons in the population, and according to the season at which the counts are made. If, for purposes of establishing a standard of comparison, one might assume that living cocoons will subsequently come to the same fate as the empty cocoons (emerged and dead), then it is possible to calculate comparable statistics independent of the age of the infestation or the time of sampling in relation to seasonal development. The principal interest here is in the proportion of the population that has participated in continuation of the infestation, and the calculation is therefore the percentage relationship of emerged cocoons to all empty cocoons. The accompanying synopsis includes such statistics for the data in Table III.

Forest type and locality	Year	Survival, %
White spruce flat, Berry Mountain Brook	1933	10
	1934	13
	1937 (live trees only)	18
	1937 (dead trees only)	17
	Average	15
Black spruce slope, Berry Mountain Brook	1932	35
	1933	32
	1934	29
	1935	26
Brandy Brook	1935	30
	1936	33
	1937	32
	1938	32
	1939 (all trees)	32
	1939 (dead trees only)	35
	1940 (all trees)	32
	1940 (dead trees only)	33
	Average	32

The difference in survival between the two forest types is highly significant, reflecting the effect of poor drainage in the white spruce flat. The uniformity of the annual estimates, particularly for the black spruce slope where the most intensive sampling was done, substantiates the basic assumption of a fate for living cocoons similar to that of their predecessors, *provided of course that no new elements of control enter the complex*. This qualification is important in respect to results obtained in New Brunswick, though not so far applying to central Gaspé where no perceptible change in the control elements has occurred between 1932 and 1940.

It may be concluded that about 15% of the population in the white spruce flat, and about 32% in the black spruce slope, survived the period of dormancy to continue the infestation.

A valuable analysis of the role of diapause in contributing to rapid termination of an outbreak of *Diprion similis* Hartig in Poland is given by Hardy (3). Diapause in cocoons surviving the winter was almost 100%, and there was no addition to the population during the summer. Meanwhile disease and cocoon parasites flourished in the dormant population, quashing the outbreak in a single year.

The only instance of extremely high diapause in the spruce sawfly within our experience, viz., 98 to 99% at Parke Reserve in 1934, had no such catastrophic consequences as those reported by Hardy. The reduction in living cocoons per square foot during the year was as follows:

	May, 1934	May, 1935	Reduction, %
Samples under white spruce	27.7	18.8	32
Samples under black spruce	13.1	8.3	37

Emergence was about 12% in 1935, and since then the infestation has followed a normal course. Based on population records over a 5-year period, survival has averaged 23%.

Due to the high degree of diapause and comparatively low survival from the period of dormancy, infestations in a typical one-generation area are moderately slow in development but persistent, being protected by the reserve in diapause from the effects of very unfavourable conditions in a particular year. The only factor that has been noted as responsible for a steady decline in population of living cocoons in a one-generation area is the severe defoliation and death of the host. Current additions to the population may fail to compensate for current depletions (emergence and mortality) when the remaining old foliage is reduced to 10% or less of the normal complement of a healthy tree. At this stage the reduced population may still be denser in relation to food supply than during the stages of population increase, and there may even be a temporary increase during an exceptionally favourable year. An illustration of this occurs in the accompanying synopsis, showing cocoon density, adult emergence, and condition of the host trees in a black spruce slope in central Gaspé.

Year	Density per square foot		Condition of host trees		
				Surviving trees**	
	Living cocoons	Emerging females	Percentage dead*	Percentage of defoliation	Percentage of twig mortality
1935	17.6	3.0	2	75	31
1936	13.5	3.8	5	85	37
1937	9.3	4.1	9	95	64
1938	13.9	3.2	19	93	52
1939	8.6	2.4	29	87	51
1940	5.3	3.6	32	88	54

NOTE: *Population counts made in June, condition of host trees checked in September. The season of 1937 was exceptionally favourable, reflected in the population of June 1938.*

** Cumulative.*

*** Ocular estimate; the slight reduction in injury to the surviving trees in later years was partly due to elimination of most severely injured trees through mortality, and partly to errors in estimating.*

The evidence obtained so far therefore indicates that without some new element of control†, the spruce sawfly in one-generation areas is likely to persist as a serious menace as long as there is host material to support it.

Diapause in a Two-Generation Area

The data on diapause in a two-generation area relate principally to south central New Brunswick. A few observations pertaining to the State of Maine are included for comparison.

† The liberation of exotic parasites is of course designed to introduce the needed element of control. Results in central Gaspé, to date, do not justify an expectation that the current infestation in that area will be significantly altered. In other areas, more recently and less severely infested, the outlook is more encouraging, due both to greater parasite effectiveness and to a virus disease which kills the developing larvae.

Duration of Diapause

The successive yearly emergence from a number of experimental lots of cocoons is shown in Table IV. Most of these lots were kept in wood-covered containers during the summer, and since it has more recently been shown that withholding moisture during the summer may result in a prolongation of diapause in New Brunswick material, it follows that the results are not entirely typical of the natural field populations. The error is on the conservative side, and therefore may be regarded as strengthening the significance of the difference between the diapause periods of populations in one-generation and two-generation areas.

TABLE IV

EMERGENCE IN SUCCESSIVE YEARS FROM LOTS OF NEW BRUNSWICK FIELD COLLECTED COCOONS

Locality	Time of collection	Original number of cocoons	Emergence in successive years					Total emergence, %
			1935	1936	1937	1938	1939	
Estey Bridge, York Co.	May, 1935	160	133	7	—	—	—	87
Estey Bridge, York Co.	May, 1936	101	—	60	26	—	—	85
Stanley, York Co.	Oct., 1935	342	—	204	53	33	—	85
South Tay, York Co.	Oct., 1935	280	—	120	87	50	—	92
South Tay, York Co.	May, 1936	125	—	74	41	—	—	92
Young's Brook, York Co.	June 4, 1936	167	—	41	69	34	—	86
Young's Brook, York Co.	May 6, 1937	414*	—	—	268	54	—	78
Young's Brook, York Co.	May 18, 1937	1420*	—	—	851	338	81	89
McNamee, Northumberland Co.	May, 1935	149	34	75	24	—	—	89
McNamee, Northumberland Co.	May, 1936	135	—	60	33	29	—	90
McNamee, Northumberland Co.	May, 1937	476	—	—	117	76	215	86
Canaan River, Queens Co.	Nov., 1936	1110*	—	—	673	128	—	72

* *These lots were kept in screen covered cages during the summer. All others were kept in wood covered cages. See text.*

The average distribution of seasonal emergence from the lots summarized in Table IV is as follows:

Year	1	2	3	Total
Number of sawflies	2635	987	466	4088
Percentage of total	64.5	24.1	11.4	100

The total emergence was 84% of the original number of cocoons, the range in individual lots, 72 to 92%.

Variations in Diapause

Data on variations in the percentage of diapause in different New Brunswick localities, based on samples collected in the spring of the year for which the record is given, appear in Table V.

TABLE V

VARIATIONS IN THE PERCENTAGE OF DIAPAUSE IN OVERWINTERED COCOONS IN DIFFERENT LOCALITIES IN NEW BRUNSWICK, 1935 TO 1940

Year	Locality	Number of cocoons	Diapause, %
1935	McNamee, Northumberland Co.	149	77
	Estey Bridge, York Co.	160	17
1936	McNamee, Northumberland Co.	135	55
	Estey Bridge, York Co.	101	41
	South Tay, York Co.	125	41
	Young's Brook, York Co.	167	75
1937	McNamee, Northumberland Co.	476	75
	Young's Brook, York Co.	1834	39
1938	Millville, York Co.	3483	8
	Acadia Expt. Sta., Sunbury Co.	148	29
1939	St. Leonard, Madawaska Co.	196	50
	McNamee, Northumberland Co.	540	19
	Blissfield, Northumberland Co.	2359	31
	Glencoe, York Co.	1789	14
	Zionville, York Co.	1008	13
	Cross Creek, York Co.	806	8
	Nashwaak, York Co.	2078	27
	English Settlement, York Co.	4675	11
	Acadia Expt. Sta., Sunbury Co.	865	23
1940	McNamee, Northumberland Co.	269 (Lot 1)	20
		1354 (Lot 2)	36
	Blissfield, Northumberland Co.	1536	10
	Nashwaak, York Co.	652	35
	Durham, York Co.	118	9
	Hampton, Kings Co.	2032	15
	Kingston, Kings Co.	1811	16

The values for the first two years are in all probability somewhat high, because not only was rainfall excluded, but partly developed individuals were not detected as the remaining cocoons, being required for subsequent emergence, were not opened in the autumn. As a generalization, from about 8 to 40% of the overwintered cocoons in south central New Brunswick (Kings, Sunbury, and York counties) remained in diapause throughout the season. Farther north, in Northumberland and Madawaska counties, the values ranged from 19 to about 70%.

Peirson and Nash (4) report that 35% of the overwintered cocoons in Aroostook County, Maine, remained in diapause during the 1939 season.

Epidemiology

The spruce sawfly was known to be distributed throughout south central New Brunswick in 1933, but only at a very low population level. Nowhere was there any noticeable defoliation. There was no apparent change in population density in several localities where plot studies were in progress during the following two or three years. Beginning in 1936 in some localities and in 1937 in others, there was a marked increase in population accompanied by defoliation injury which in some woodlands became severe within two years of the first sign of larval feeding.

TABLE VI

COCOON POPULATION AND MORTALITY, SAMPLE PLOTS IN NEW BRUNSWICK. AVERAGES TO NEAREST 0.1 EXCEPT WHERE VERY SMALL

Locality	Year	Square feet in sample	Cocoons per square foot						Total mortality, %
			Living	Emerged	Chewed	Insect-killed	Dead	Total	
Hanwell Road, York Co.	Oct., 1934	160	0.4	0.4	0.1	0.1	0.02	1.1	21
	Oct., 1935	160	0.1	0.7	0.3	0.3	0.05	1.5	46
Fredericton, York Co.	Oct., 1934	76	0.3	0.4	0.1	0.1	0	0.9	18
	Oct., 1935	72	0.2	0.8	0.2	0.2	0.04	1.4	30
McNamee, Northumberland Co.	Oct., 1934	32	1.0	0.2	0.2	0.5	0	1.8	36
	May, 1935	196	0.8	0.4	0.3	0.3	0.04	1.8	37
	May, 1936	196	0.7	0.5	0.7	0.4	0.1	2.3	50
	May, 1937	196	2.6	0.9	0.9	0.5	0.1	5.0	29
	May, 1938	196	5.8	1.2	1.4	1.4	0.1	9.9	29
Estey Bridge, York Co.	May, 1935	272	0.6	0.7	0.1	0.03	0.03	1.4	11
	May, 1936	280	0.4	1.1	0.3	0.05	0.03	1.8	20
Royal Road, York Co.	Oct., 1935	200	1.7	1.1	0.06	0.15	0.04	3.1	8
	Oct., 1936	200	1.0	1.9	0.3	0.2	0.09	3.4	17
	Oct., 1937	248	8.9	2.4	0.3	0.3	0.2	12.1	7
	Oct., 1938	200	15.5	7.7	2.3	0.9	1.2	27.6	15
	Oct., 1939	200	6.7	10.3	3.9	1.7†	3.5	26.1	35
	Oct., 1940	188	0.2	11.9	2.2	4.0†	2.5	20.8	41
Canaan River, Queens Co.	Oct., 1936	196	12.3	2.9	2.1	1.3	0.45	19.1	20
	Oct., 1937	200	20.8	7.8	3.6	2.7	0.9	35.8	19
	Oct., 1938	200	21.8	9.8	10.4	2.9	0.9	45.8	31
	Oct., 1939	200	10.4	16.3	18.6	3.2	2.6	51.1	48
Young's Brook, York Co.	May, 1937	200	8.0	2.4	0.9	0.6	0.6	12.5	17
	May, 1938	200	16.7	4.5	3.0	1.7	0.7	26.6	20
	May, 1939	200	24.0	12.6	15.8	2.7	7.9*	63.0	42
	May, 1940	200	6.5	16.4	19.8	4.3**	6.5	53.5	57
Acadia Expt. Sta., Sunbury Co.	May, 1938	200	2.3	1.8	1.6	1.6	0.06	7.3	44
	May, 1939	200	5.2	2.6	2.3	2.7	0.3	13.1	40
	May, 1940	200	1.4	4.5	3.2	5.0†	0.4	14.5	59

* *Many eonymphs were diseased at time of spinning, dying later.*

** *Includes 0.5 cocoons per square foot killed by parasites.*

† *Includes 0.3 cocoons per square foot killed by parasites.*

Population data for a number of plots appear in Table VI. Mortality within the cocoon was quite variable between plots and years, but on the whole, predatory mammals were less important than in Gaspé; predatory insects (elaterids) were about equally important as in Gaspé, except in the McNamee and Acadia Experiment Station plots, where their relative effectiveness was unusually high; other causes of mortality in the cocoon, barring parasites and disease in the later years, were of only minor importance.

TABLE VII

SURVIVAL RATES, SAMPLE PLOTS IN NEW BRUNSWICK

Locality	Year of sampling							Average*
	1934	1935	1936	1937	1938	1939	1940	
Hanwell Road	57	50	—	—	—	—	—	52
Fredericton	67	67	—	—	—	—	—	67
McNamee	25	40	31	37	29	—	—	32
Estey Bridge	—	87	79	—	—	—	—	82
Royal Road	—	79	79	75	64	53	58	60
Canaan River	—	—	43	52	41	40	—	43
Young's Brook	—	—	—	53	45	32**	35 (Spring) 31 (Fall)†	35
Acadia Expt. Sta.	—	—	—	—	36	33	34	34

* *Based on totals from all years' sampling.*

** *Reduction due to death after spinning of 1938 generation material affected by disease.*

† *Samples totalling 244 square feet taken in general area but not on the standard plot.*

Estimates of the percentage survival (emerged cocoons in relation to all empty cocoons) for the different plots are shown in Table VII. The survival rate varied widely between different localities, but variations in different years in a given locality were of a minor degree except in the latest years; disease and cocoon parasites, new elements in the control complex, caused a considerable reduction in survival in the cocoon stage in the Royal Road and Young's Brook plots. On the whole, survival in New Brunswick was at least equal to that in the most favourable locality in central Gaspé, and in most of the New Brunswick plots there was a marked superiority.

Similar estimates for localities in central to northern Maine, based on 1939 population data obtained by American entomologists, appear in the synopsis.

Locality	Source of data	Survival rate		
		Spring samples	Fall samples	Average
Township 12, Range 16	Brown *et al.* (2)	25	20	23
Township 30	Brown *et al.* (2)	60	56	58
Aroostook County	Peirson and Nash (4)	61	53	58

With the exception of the first locality, the estimates fall within the range of variations noted for New Brunswick localities.

The infestations in New Brunswick, and in the northeastern states as well, were often quite localized but built up rapidly. Severe defoliation injury frequently resulted within a year or two of the first signs of feeding. The reduction in population level in 1939 and 1940 was due to a combination of circumstances, all unfavourable to the insect. Only small reserves of dormant eonymphs were maintained during the summer season, and there was also a considerable emergence from earlier cocoons of the first generation. Each year a destructive virus disease appeared among the feeding larvae in July, killing a very high proportion of the then existing larval population and practically all larvae ensuing from subsequently emerging adults. Virtually all emergence occurring after June was therefore non-productive. Meanwhile introduced parasites, having built up to effective populations in many localities in south central New Brunswick, were added to the complex of factors affecting the dormant sawflies in the ground.

Diapause and Epidemiology Farther South

The following observations relating to southern New Hampshire and Vermont, tentatively considered in the absence of field records as an area in which a partial third generation probably occurs, are based almost entirely on information supplied by officers of the United States Bureau of Entomology and Plant Quarantine, New Haven, Conn. (2).

Less than 1% of the overwintered cocoons at Wilmington, Vt., persisted in diapause during the 1938 season. This was also the condition in 1939, less than 1% of the overwintered population being unpupated by June 7 (1). Estimates of the degree of diapause at Marlboro, Vt., in 1939, ranged from less than 1% (about 2600 cocoons in soil cages) to about 15% (comparison of spring and autumn population data). At Dublin, N.H., diapause was estimated at 10 to 15%.

Diapause in cocoons of the first generation at Marlboro in 1939 was 12 to 13%.

From these observations it may safely be concluded that large proportions of the overwintered and first generation cocoons produce adults for participation in the seasonal development. During the early part of the season, at least, there is evidently a very small reserve population in diapause.

Survival of cocoons to the time of adult emergence, as based on extensive field counts in the spring and autumn of 1939, averaged 57% at Dublin and 70% at Marlboro.

The infestations in this general area have been more or less localized, but in a number of cases developed with great rapidity to population densities far in excess of those experienced in Gaspé or New Brunswick. Extensive counts of the cocoon population in a woodland near Dublin in 1939 gave a total density of about 160 per square foot; of these, about 85 were living cocoons

in May. As many as 90,000 larvae were counted climbing a single tree during part of the first generation period. In midsummer and later disease swept through the larval population and left practically no survivors. The living cocoons in the autumn were reduced to 11 per square foot.

Extensive samples were also taken at Marlboro in 1939. Here the cocoon density was even greater, about 180 per square foot, but the infestation had apparently been greatly reduced in 1938, since only two cocoons per square foot were living in May, 1939. This was further reduced to 0.4 in October.

Severe defoliation in scattered woodlands throughout the area resulted from the huge populations, but apparently there has been little or no mortality of spruce to date.

The rapid population increases in this area apparently are due to the combination of *(a)* small degree of diapause in overwintered and first generation cocoons, *(b)* fairly high survival to the time of adult emergence, and *(c)* increased number of seasonal generations. At the same time the population is very unstable because of small reserves of cocoons in diapause, and susceptible to violent reduction by factors, such as disease, affecting the feeding larvae.

Discussion

The question of intraspecific differences is of particular interest in this study, and observations bearing on the probable origin of diversity within the species, its perpetuation, and the interaction of climate and diapause as affecting the character of populations in different regions require some further comment.

The origin and subsequent genetic history of the obligatorily parthenogenetic spruce sawfly have been discussed by Smith (5). From a cytological survey of related species of the genus *Diprion*, Smith concludes that the basic chromosome number is seven, and therefore, that both the facultative (6-chromosome) and obligatory (7-chromosome) species formerly included in *polytomum*, arose from a common 7-chromosome prototype, which was a facultatively parthenogenetic (bisexual) species. It is plausible to assume that the obligatory form was more or less heterozygous at origin. Starting out with a certain degree of heterozygosity and possibly accentuated by subsequent mutation, it is inevitable that the species become split into races, due to the self-fertilizing mechanism, inhibition of interbreeding between females and the rare males (infertile triploids would probably result), and the barrier between crossing of the facultative and obligatory forms provided by the chromosome differences. The process of developing a number of homozygous lines would be gradual, but progressive with each generation. Smith found differences in chromosome length, pigmentation of the fifth instar head capsule and gross cocoon weight of European obligatory material, which suggested the existence of distinct lines or races.

In our studies with Canadian obligatory material, we have found differences in the tendency towards diapause and in the proportion of males in pure lines,

which appear explicable only on the basis of racial physiological differences. Higher proportions of males occurred in emergent lines than in diapause lines or in field populations. Smith (5) suggests that variations in male production in different lines is probably due to inherent differences in the factors controlling the speed of budding off of the second polar body from the female pronucleus at the time the latter begins to sink into the yolk; a rapid budding off would result in a male through the failure of auto-fertilization. The higher male production in the laboratory than in the field may be explained as due to the accelerative effect of the higher temperature upon the process of budding off, within limits imposed by the inherent factors controlling the process.

The varied behaviour, with respect to diapause, of different lines reared in the incubator suggests corresponding genetic differences in the factors controlling development, but the modus operandi of such factors, in contrast to those of sex determination, is entirely unknown.

The occurrence of emergent and diapause lines in both one-generation and two-generation areas, though in different proportions, indicates that climate has had only a partially selective action in fashioning the composition of the population from the various elements within the species. However, it seems significant that no strongly emergent lines have been found in Gaspé material: at most, only lines that were partially emergent for a very few generations, then terminated altogether due to diapause. From the rearings of emergent lines transferred to Gaspé it is clear that there would be a partial survival of such stock in a one-generation area by virtue of diapause induced by environmental causes.

At the other extreme, diapause lines would appear to have no intrinsic handicap in an area favourable to the development of two or more annual generations, but in view of their slower rate of increase they would in time be greatly outnumbered by emergent strains that made full use of the environmental possibilities.

The environmental causes that induce diapause in emergent stock with advance of the season are obscure, but the adaptive value of the phenomenon is apparent in view of the fact that development is arrested in cocoons of emergent stock spun in late August, under temperature and moisture conditions in the soil about as favourable as those of May and early June, when development is resumed.

Summary

1. The European spruce sawfly is favoured in America by the parthenogenetic mode of reproduction and by the freedom of attack from native parasites. It is well adapted, by means of variations in the diapause phenomenon, to climatic conditions over a wide area and is probably capable of adapting itself to all areas in which spruce grows.

2. The life history and developmental stages within the cocoon are described. The newly spun larva (eonymph) is most commonly affected by diapause and may remain dormant for long periods, up to seven years or more. Eonymphs

in diapause are resistant to factors (except, of course, predatism or parasitism) that cause mortality in later developmental stages.

The advanced larva preceding the pupa (pronymph) may occasionally go into a true diapause, though usually of only a few months' duration.

3. Studies are described which indicate that there are different "lines" within the species, with respect to the inherent tendency towards diapause or continued development; and that the population in southern areas (two or more annual generations) is composed predominantly of "emergent" lines, while the population in northern areas (one annual generation) is composed predominantly of "non-emergent" or "diapause" lines, diapause intervening after a single generation even at favourable environmental conditions.

4. Diapause may be induced in the members of an emergent line by sub-optimal conditions, such as low fluctuating temperature and unfavourable foliage. However, there is no intimate relation between the percentage of diapause in the larvae of an emergent population, and any one of the three factors, temperature, moisture, or food. The only conclusion that can be drawn is that emergent populations are evidently highly sensitive to environmental changes associated with advance of the season. The result is that diapause in the larvae maturing at successive intervals becomes complete at a time when the season is so far spent that a further generation could not develop to maturity. This is the manner by which the insect adapts itself to areas in which more than one generation is possible.

The character of the population in different areas seems to have been fashioned out of the various elements within the species, by the interaction of climatic and genetic factors with respect to diapause and survival.

5. On the basis of biologic and climatic data the eastern part of Canada and adjoining territory in the United States have been divided into zones, representing one-, two-, and three-generation areas; intermediate transitional zones are also indicated. Though much of the zonation is admittedly tentative, it will serve to focus attention upon various aspects of the bioclimatology that require further study.

6. Laboratory and field studies of the factors influencing the resumption of development are described. With Gaspé (one-generation) material, there was typically a progressive response to favourable developmental conditions as the period of cold rest was extended to three or four months. New Brunswick (two-generation) material gave a high response after two months of cold rest. The degree of cold was unimportant, provided it was below the threshold of development.

An incubation temperature of 74° to 75° F. promoted a higher development than one of 65°, but there was little difference in the percentage of development over the limits of 65° to 45°, the latter being close to the threshold of development.

The percentage of development after appropriate cold rest and at an incubator temperature of 74° to 75° was in all cases much lower in unsaturated

atmosphere than at 100% relative humidity. Contact water was not essential to secure very high development in New Brunswick material incubated at this temperature, but in most cases it was necessary to secure high development in Gaspé material. Effective methods of providing contact water include incubation on moist sand or filter paper, frequent momentary dippings, or immersion for one to three days.

The cocoon has an important bearing on water exchanges. It appears to be the only mechanism possessed by the larva to conserve its water supply, since a fatal loss of water occurs when the larvae are removed from the cocoon. (It may have other important functions as well, e.g., respiration, since naked larvae at 100% relative humidity die before water loss reaches fatal proportions.) The protection against water loss afforded by the cocoon is only relative, however, since the rate of loss is nearly proportional to time and saturation deficiency.

The larva absorbs water taken up by the cocoon wall at contact. Absorption is slow since the inner lining of the cocoon is relatively impervious. Preliminary drying may cause a greater water loss than can be compensated for by a brief contact with water, and drying after contact removes the water held in the cocoon wall.

In controlled experiments there was a progressive decrease in the percentage of development in relation to the degree of drying either before or after contact of the cocoons with water.

Temperature fluctuations within the limits experienced in the natural environment appear to have little or no direct influence on the percentage of development in overwintered cocoons. Moisture deficiency in May and June may greatly retard development in the overwintered cocoons in south central New Brunswick, but the insects in diapause respond to rainfall later in the season. Although development in the overwintered cocoons in Gaspé may be inhibited by deficient moisture in the spring, the population does not respond to any appreciable extent to plentiful moisture later in the season (July onwards); it is therefore possible to estimate percentage of seasonal development several weeks before emergence begins.

The characteristically small percentage of development in populations of one-generation areas can only be attributed to the inherent tendency towards diapause, the temperature conditions in the natural habitat failing as a marked stimulus for resumed development.

7. The character of sawfly infestations depends upon several factors which vary in degree throughout the distribution range in North America, viz.: (1) degree of diapause in overwintered cocoons; (2) duration of diapause; (3) number of seasonal generations; and (4) survival of cocoons to the time of adult emergence. Data showing the variability of these factors in a one-generation, a two-generation, and an area probably having a partial third generation, are included, and the nature of the infestations is described. Infestations in northern localities develop slowly but persistently, those in southern localities have violent oscillations in population level.

8. Reproductive capacity of the spruce sawfly is correlated with size, and both are influenced by feeding conditions during larval development, being greater on white spruce than on black spruce. Under very satisfactory conditions, reproductive capacity is approximately 60, but reductions of 30 to 40% may result from food shortage.

The influence of prolonged diapause (four to five years) upon reproductive capacity is small and masked by errors of random sampling. The apparent lack of influence is explicable on the basis of very low metabolic demands upon the stored nutriments during the period of diapause.

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